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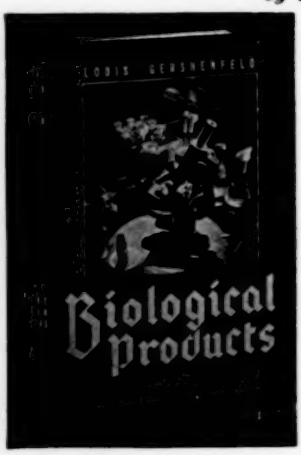

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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On the Biological Assay of Digitalis by the Over-Night Frog Method

*By M. G. Allmark and C. A. Morrell**

In biological assays which depend on a quantal response the use of dosage-response curves is becoming widely adopted. This is particularly true in the assay of digitalis when frogs are used as the test animals. Dosage-response curves offer a means of overcoming difficulties in interpretation of results which are due to the variability of animals (1), and also give the necessary data for computing the errors of an assay (2, 3). The slope and position of these curves give requisite information for the design of a test, for theoretically, if it can be shown that the slope does not change significantly, only one dose each of the standard and the unknown are necessary (2, 4), and if the position is constant, the standard may be dispensed with after a preliminary determination of the "characteristic" curve.

Simplicity is considered of great importance in the design of any method of assay intended for general routine use. The ease with which the results of a one-dose assay may be calculated is in itself a strong recommendation for its adoption, provided, of course, that it is as accurate as other methods.

* Laboratory of Hygiene, Department of Pensions and National Health, Ottawa, Canada.

The results from assays of samples by both one-dose and multiple-dose procedures as reported in this paper indicate that a method using a single dose each of the standard and sample, and based on a "characteristic" curve, may be used for the assay of digitalis by the over-night frog method. The results obtained from the assay of samples by this method check very closely with those of the more laborious three-dose procedure.

EXPERIMENTAL

In previous papers (5, 6) the care of the frogs, the type of apparatus used and the preparation of dilutions were described. For the construction of regression lines in Tables I, II and III, the Canadian Standard Digitalis, Lot No. 1135, and Canadian Standard Ouabain, Lot No. 330, were used. For the experiments in Table I exactly 0.6 Gm. of powder was extracted in each Soxhlet for two hours, using absolute alcohol as the menstruum. Various methods of extracting the Canadian Standard powder were employed for the experiments in Table II. The dilutions for injection were made up so that the dose per Gm. of frog was contained in 0.02 cc. of a liquid having 20 to 25 per cent by volume of ethyl alcohol. All frogs used for this investigation had been kept in concrete storage tanks with running water at approximately 10 ± 5° C. from two weeks to three months previous to use.

A.J.H.

Table I.—Data Used in Computing Regression Lines for Digitalis

Powders extracted by Soxhlet method only

Date	Dose in Gm. per Gm. of Frog × 10^{-4}	% Mortality	Sex	[b] Slope of Regression Line
1938				
Oct. 24	41	40.0	M	17.6
	49	86.7		
Oct. 28	41	13.3	M	20.0
	49	66.7		
Nov. 2	41	16.7	M	23.4
	49	80.0		
Nov. 4	41	30.0	M	16.2
	49	76.7		
Nov. 8	41	23.3	M	20.3
	49	80.0		
Nov. 10	35	3.3	M	15.5
	40	3.3		
	46	23.3		
	53	63.3		
	61	83.3		
	70	100.0		
Nov. 14	35	3.3	M	13.0
	40	13.3		
	46	33.3		
	53	60.0		
	61	86.7		
	70	100.0		
Nov. 16	40	6.7	M	11.4
	46	20.0		
	53	53.3		
	61	70.0		
Nov. 16	40	13.3	M	11.9
	53	63.3		
Nov. 18	41	20.0	M	12.0
	49	53.3		
Nov. 18	40	6.7	M	19.9
	46	10.0		
	53	63.3		
	61	93.3		
	70	100.0		

Note: 30 frogs were used on each dose.

Table II.—Data Used in Computing Regression Lines for Digitalis

Powders extracted by various methods

Date	Dose in Gm. per Gm. of Frog × 10^{-4}	% Mortality	Sex	[b] Slope of Regression Line
1938				
Oct. 11	26	6.7	F	10.0
	31	20.0		
	35	6.7		
	40	23.3		
	46	53.3		
	53	76.7		
Oct. 13	26	0.0	F	13.3
	31	3.3		
	35	13.3		
	40	53.3		
	46	63.3		
	53	90.0		
	61	100.0		

Table II.—(*Continued*)

Oct. 18	26	0.0	M	8.8
	31	3.3		
	35	26.7		
	40	50.0		
	46	60.0		
	53	80.0		
	61	86.7		
	70	100.0		
Oct. 19	26	3.3	M	13.0
	31	3.3		
	35	10.0		
	40	26.7		
	46	46.7		
	53	86.7		
	61	96.7		
	70	100.0		
Oct. 20	31	0.0	M	13.9
	35	3.3		
	40	23.3		
	46	60.0		
	53	73.3		
	61	96.7		
	70	96.7		
Oct. 24	41	33.3	M	11.1
	49	66.7		
Oct. 24	41	43.3	M	10.2
	49	73.3		
Oct. 24	41	26.7	M	14.8
	49	70.0		
Oct. 28	41	23.3	M	17.5
	49	73.3		
Oct. 28	41	16.7	M	12.5
	49	50.0		
Oct. 28	41	26.7	M	10.3
	49	56.7		
Nov. 2	41	30.0	M	21.1
	49	86.7		
Nov. 2	41	43.3	M	18.7
	49	90.0		
Nov. 2	41	40.0	M	15.8
	49	83.3		
Nov. 4	41	16.7	M	12.5
	49	50.0		
Nov. 4	41	26.7	M	16.0
	49	73.3		
Nov. 4	41	30.0	M	13.5
	49	70.0		
Nov. 22	40	23.3	M	16.7
	46	46.7		
	53	93.3		
	61	96.7		
	70	100.0		
Nov. 22	43	50.0	M	10.7
	53	83.3		
Nov. 24	41	36.7	M	10.0
	49	66.7		
Dec. 20	41	20.0	M	22.1
	45	40.0		
	49	80.0		

Table II.—(*Continued*)

1939				
Jan. 26	28	16.7	F	8.7
	31	46.7		
	34	36.7		
	38	66.7		
Jan. 27	28	13.3	F	9.6
	31	40.0		
	34	36.7		
	38	63.3		
Jan. 30	28	15.0	F	15.7
	31	23.3		
	35	61.6		
	39	86.6		
Feb. 1	28	46.7	F	15.0
	31	80.0		
	35	86.7		
	39	100.0		

Note: Thirty frogs were used on each dose, except on December 20, 1938, and January 30, 1939, when 10 and 60 frogs were used, respectively.

Table III.—Data Used in Computing Regression Lines for Ouabain

Date	Dose in Gm. per Gm. of Frog × 10^{-4}	% Mortality	Sex	$\hat{b}$ Slope of Regression Line
1939				
Mar. 10	25	36.7	M	22.8
	27	50.0		
	29	90.0		
Mar. 16	20.5	0.0	M	17.2
	23	20.0		
	26	70.0		
	29	73.3		
Mar. 17	23	10.0	M	32.7
	25	36.7		
	27	83.3		
Mar. 22	23	3.3	M	22.5
	25	40.0		
	27	70.0		
	29	73.3		
Oct. 5	26	10.0	M	16.6
	29	43.3		
	32	60.0		
Oct. 6	26	60.0	M	19.4
	29	93.3		
	34.5	100.0		
Oct. 27	26	4.0	M	29.1
	30	60.0		
	34.5	96.0		
Oct. 30	26	16.7	M	24.0
	30	76.0		
	34.5	96.7		
Nov. 7	26	63.3	M	13.5
	30	83.3		
	34.5	98.3		

Table III.—(*Continued*)

Nov. 10	20	13.3	F	21.9
	23	53.3		
	26	93.3		
	30	96.7		
Nov. 13	20	3.3	F	21.6
	23	20.0		
	26	56.7		
	30	96.7		
Nov. 14	20	0.0	F	24.1
	23	20.0		
	26	63.3		
	30	96.7		

Note: Thirty frogs were used on each dose except as follows: Oct. 27, 1939, 25 frogs were used on each dose; Oct. 30, 1939, 25 frogs were used on the middle dose; Nov. 7, 1939, 60 frogs were used on the high dose.

Regression Lines.—Tables I, II and III present the data from which the individual regression lines were obtained. The methods used in computing the parameters for the curves were those described by Bliss (3).

Application of Bliss' equation 20*a* (7) demonstrated that the apparent differences in the slopes of the lines for digitalis, Tables I and II, and for ouabain, Table III, were not significant, and could have occurred as a result of sampling variation.

The position of the lines for digitalis and ouabain, however, were shown by a modification (8) of Bliss' equation 19 (7) to differ to a significant degree. The use of a standard with each test is, therefore, necessary. Data from Table II were not used in computing chi-square for position because several methods of extracting the Canadian Standard powder were used in these experiments.

Since the slopes of the separately determined lines did not differ significantly from their means, composite lines for both digitalis and ouabain were computed by methods described elsewhere (3). The composite regression coefficient for digitalis was found to be 13.1, for ouabain 21.2 and the disagreement between these two values was found to be significant.

Comparison of Methods.—In Table IV the results of 50 assays are shown. Three doses each of the standard and samples have been used and potencies were computed by the procedure recommended by Gaddum (2) and Bliss (3, 7), and by curve numbers (5, 6, 9). In the former case the slope and position of each regression line were computed separately, while in the latter the slope of the line already determined for the standard was employed, and each experiment treated as three separate assays; the low, middle and high doses of the unknown being compared with the corresponding doses of the standard. The figures given in Columns 7 and 8 of the table are in each instance the arithmetic mean of the three results calculated in this way when a standard curve for digitalis (Curve *D*) and for ouabain (Curve *O*) were used. The slopes for Curve *D* and Curve *O* were obtained from the data shown in Tables I, II and III.

Table IV.—Comparison of Methods

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose Method One-Dose Curve *D*	% Variation from Three-Dose Method One-Dose Curve *O*
1939									
Oct. 27	Std.	2.5	25	52.0					
		2.9	25	52.0					
		3.4	25	88.0					
Oct. 27	U	5.3	25	12.0	42	43	45	2.4	7.1
		6.2	25	40.0					
		7.2	25	88.0					
Dec. 7	Std.	2.7	30	23.3					
		3.2	30	40.0					
		3.9	30	93.3					
Dec. 7	V	2.5	31	48.4	122	117	111	4.1	9.0
		3.1	30	76.7					
		3.9	30	96.7					
Dec. 8	Std.	2.7	20	20.0					
		3.2	20	40.0					
		3.9	25	76.0					
Dec. 8	V	2.5	25	40.0	122	118	112	3.3	8.2
		3.1	20	60.0					
		3.9	25	96.0					
1940									
Jan. 6	Std.	2.51	10	20.0					
		3.16	10	70.0					
		3.98	10	100.0					
Jan. 6	A	1.78	10	0.0	129	135	137	4.7	6.2
		2.24	10	60.0					
		2.82	10	90.0					
Jan. 8	Std.	2.51	15	46.7					
		2.82	15	33.3					
		3.16	15	80.0					
Jan. 8	A	2.00	15	40.0	137	135	131	1.5	4.4
		2.24	15	66.7					
		2.51	15	100.0					
Jan. 10	Std.	2.51	15	40.0					
		2.82	15	60.0					
		3.16	15	93.3					
Jan. 10	A	2.00	15	40.0	130	130	128	0.0	1.5
		2.24	15	73.3					
		2.51	15	100.0					
Feb. 8	Std.	2.5	15	33.3					
		3.1	15	40.0					
		3.9	15	80.0					
Feb. 8	B	1.9	15	6.7	114	119	122	4.4	7.0
		2.4	15	26.7					
		3.0	15	73.3					
Feb. 8	C	1.0	15	33.3	269	266	263	1.1	2.2
		1.2	15	46.7					
		1.45	15	86.7					
Feb. 9	Std.	2.5	15	20.0					
		3.1	15	60.0					
		3.9	15	100.0					
Feb. 9	B	1.9	15	26.7	118	127	128	7.6	8.5
		2.4	15	40.0					
		3.0	15	78.6					
Feb. 9	C	1.0	15	13.3	253	257	255	1.6	0.8
		1.2	15	73.3					
		1.45	15	86.7					
Apr. 4	Std.	2.24	15	13.3					
		2.51	15	13.3					
		2.82	15	33.3					

Table IV.—(*Continued*)

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose Method One-Dose Curve *D*	% Variation from Three-Dose Method One-Dose Curve *O*
Apr. 4	D	2.24	15	6.7	100	100	99	0.0	1.0
		2.51	15	13.3					
		2.82	15	46.7					
Apr. 4	S	2.24	15	26.7	125	122	112	2.4	10.4
		2.51	15	66.7					
		2.82	15	80.0					
Apr. 5	Std.	2.51	15	26.7					
		2.82	15	26.7					
		3.16	15	66.7					
Apr. 5	D	2.51	15	33.3	109	106	105	2.8	3.7
		2.82	15	53.3					
		3.16	15	73.3					
Apr. 5	S	2.24	15	26.7	125	124	120	0.8	4.0
		2.51	15	66.7					
		2.82	15	100.0					
Apr. 8	Std.	2.51	15	20.0					
		2.82	15	53.3					
		3.16	15	93.3					
Apr. 8	D	2.51	15	26.7	100	99	99	1.0	1.0
		2.82	15	66.7					
		3.16	15	73.3					
Apr. 8	T	2.24	15	26.7	112	111	111	0.9	0.9
		2.51	15	73.3					
		2.82	15	66.7					
Apr. 11	Std.	2.51	15	26.7					
		2.82	15	46.7					
		3.16	15	80.0					
Apr. 11	D	2.51	15	46.7	98	102	102	4.1	4.1
		2.82	15	46.7					
		3.16	15	73.3					
Apr. 11	T	2.24	15	13.3	109	110	111	0.9	1.8
		2.51	15	53.3					
		2.82	15	80.0					
Apr. 19	Std.	3.16	15	33.3					
		3.55	15	53.3					
		3.98	15	86.7					
Apr. 19	E	2.82	15	33.3	103	105	108	1.9	4.9
		3.16	15	53.3					
		3.55	15	46.7					
Apr. 19	F	1.41	15	40.0	233	233	230	0.0	1.3
		1.59	15	73.3					
		1.78	15	86.7					
Apr. 19	G	2.24	15	13.3	122	121	125	0.8	2.5
		2.82	15	46.7					
		3.16	15	73.3					
Apr. 19	H	1.26	15	33.3	253	254	253	0.4	0.0
		1.41	15	60.0					
		1.59	15	86.7					
May 8	Std.	3.16	15	46.7					
		3.55	15	60.0					
		3.98	15	66.7					
May 8	M	1.78	15	20.0	167	164	162	1.8	3.0
		2.24	15	80.0					
		2.82	15	86.7					
May 9	Std.	3.16	15	60.0					
		3.55	15	53.3					
		3.98	15	86.7					
May 9	M	1.78	15	26.7	153	161	167	5.2	9.2
		2.00	15	53.3					
		2.24	15	60.0					

Table IV.—(*Continued*)

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose — One-Dose Curve *D*	Method — One-Dose Curve *O*
May 9	N	1.78	15	73.3	212	193	187	9.0	11.8
		2.00	15	86.7					
		2.24	15	86.7					
May 9	O	1.78	15	66.7	187	181	180	3.2	3.7
		2.00	15	66.7					
		2.24	15	80.0					
May 17	Std.	3.16	15	53.3					
		3.55	15	73.3					
		3.98	15	80.0					
May 17	M	1.78	15	33.3	147	160	167	8.8	13.6
		2.00	15	46.7					
		2.24	15	60.0					
May 17	N	1.59	15	26.7	166	177	185	6.6	11.4
		1.78	15	46.7					
		2.00	15	60.0					
May 20	Std.	3.16	15	46.7					
		3.55	10	80.0					
		3.98	15	86.7					
May 20	M	1.78	15	33.3	147	153	163	4.1	10.9
		2.00	10	30.0					
		2.24	15	60.0					
May 20	N	1.59	15	13.3	181	179	187	1.1	3.3
		1.78	10	60.0					
		2.00	15	80.0					
May 20	O	1.59	15	60.0	186	188	193	1.1	3.8
		1.78	10	50.0					
		2.00	15	73.3					
May 20	Std	2.00	15	26.7					
		2.24	15	60.0					
		2.51	14	42.9					
May 20	P	2.00	14	35.7	101	102	101	1.0	0.0
		2.24	12	33.3					
		2.51	12	66.7					
May 20	Q	2.82	14	35.7	68	70	71	2.9	4.4
		3.16	15	33.3					
		3.55	15	53.3					
May 21	Std.	2.00	15	6.7					
		2.24	15	53.3					
		2.51	15	53.3					
May 21	P	2.00	15	13.3	98	99	100	1.0	2.0
		2.24	15	33.3					
		2.51	15	53.3					
May 21	Q	2.82	15	20.0	68	70	71	2.9	4.4
		3.16	15	33.3					
		3.55	15	40.0					
May 22	Std.	2.24	15	40.0					
		2.51	14	57.1					
		2.82	15	60.0					
May 22	P	2.24	15	40.0	107	107	105	0.0	1.9
		2.51	14	71.4					
		2.82	14	85.7					
May 22	Q	3.16	15	0.0	66	71	69	7.6	4.5
		3.55	14	35.7					
		3.98	14	78.6					
July 9	Std.	5.62	15	33.3					
		7.03	15	66.7					
		8.78	15	100.0					
July 9	I	4.47	15	13.3	107	110	115	2.8	7.5
		5.62	15	33.3					
		7.03	15	86.7					

Table IV.—(*Continued*)

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard Method: Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose Method: One-Dose Curve *D*	One-Dose Curve *O*
July 9	J	1.78	15	13.3	259	270	285	4.2	10.0
		2.24	15	26.7					
		2.82	15	80.0					
July 9	K	4.47	15	26.7	127	125	125	1.6	1.6
		5.62	15	73.3					
		7.03	15	100.0					
July 9	L	1.78	15	33.3	339	346	331	2.1	2.4
		2.24	15	93.3					
		2.82	15	86.7					
July 10	Std.	5.62	15	20.0					
		7.03	15	73.3					
		8.78	15	100.0					
July 10	I	4.47	15	6.7	112	113	117	0.9	4.5
		5.62	15	53.3					
		7.03	15	86.7					
July 10	J	1.78	15	13.3	261	285	297	9.2	13.8
		2.24	15	40.0					
		2.82	15	66.7					
July 10	K	4.47	15	46.7	138	140	134	1.4	2.9
		5.62	15	86.7					
		7.03	15	100.0					
July 10	L	1.78	15	33.3	302	322	320	6.6	6.0
		2.24	15	66.7					
		2.82	15	80.0					
Aug. 16	Std.	2.51	15	6.7					
		3.16	15	53.3					
		3.98	15	80.0					
Aug. 16	R	2.51	15	13.3	81	91	95	12.3	17.3
		3.16	15	20.0					
		3.98	15	40.0					
Aug. 19	Std.	3.16	15	20.0					
		3.98	15	33.3					
		5.01	15	86.7					
Aug. 19	R	3.16	15	26.7	98	99	99	1.0	1.0
		3.98	15	33.3					
		5.01	15	73.3					
Aug. 21	Std.	3.16	15	13.3					
		3.98	15	53.3					
		5.01	15	80.0					
Aug. 21	R	3.16	15	13.3	95	97	98	2.1	3.2
		3.98	15	46.7					
		5.01	15	66.7					
Aug. 23	Std.	2.82	15	20.0					
		3.55	15	26.7					
		4.47	15	60.0					
Aug. 23	W	2.82	15	6.7	74	89	93	20.3	25.7
		3.55	15	13.3					
		4.47	15	26.7					
Aug. 27	Std.	3.16	10	40.0					
		3.98	7	57.1					
		5.01	9	100.0					
Aug. 27	R	3.16	10	20.0	92	98	98	6.5	6.5
		3.98	9	66.7					
		5.01	10	80.0					

In nearly every test 15 frogs were injected with each dose. The procedure outlined in the United States Pharmacopœia XI, page 397, was followed in preparation of extracts, and the alcohol content of the material to be injected was adjusted to between 20 and 25 per cent by volume. The doses were contained in 0.02 cc., except on April 4th and 5th when the volume was 0.01 cc. Several standards were used for these comparisons.

Table V presents an analysis of the results given

in Table IV. Differences in potencies computed by the different methods from the same experimental data are shown. Figures obtained by Bliss' method of calculation (3, 7) are used as a basis of the comparison. The true potency of the samples was not known except in one case, and it is therefore impossible to judge which method gave the more accurate results. Differences between the results obtained from the methods are all that can be shown here.

Table V.—Analysis of Results in Table IV

Per Cent Difference from the Three-Dose Method	Percentage of Assays by One-Dose Method	
	Curve *D*	Curve *O*
0–5	78.0	62.0
5–10	18.0	22.0
Over 10	4.0	16.0

The results are classified according to the difference in the potencies when calculated by curve numbers and when computed by the longer procedure of the three-dose method. There was 10 per cent or less variation from the results of the three-dose method in 96 per cent of the assays using Curve *D*, and in 84 per cent when Curve *O* was used. When the variation was greater than 10 per cent the mortalities for some of the doses were low or high. If all of the mortalities fall between 20 and 80 per cent for both standard and sample the agreement between the methods will be very close.

The actual potency of Sample *W*, August 23, 1940, Table IV, was known to be 96 per cent of the standard, and the result by the one-dose method was much closer to this value than by the three-dose procedure.

It should be pointed out that if these experiments had been designed as routine assays by the one-dose method, all of the frogs would have been injected with the middle doses shown in the table. The greater weights obtained by having the mortalities for all groups of animals close to 50 per cent would reduce the errors to a minimum.

If the slope of the composite curve for digitalis be taken as 13.1, and for ouabain 21.2, Tables VI and VII show the effect of the number of animals, the per cent response and the slope of the regression line on the limits of error when assaying digitalis

Table VI.—Limits of Error Computed for One-Dose Method, Digitalis Curve

No. of Frogs Used on Standard and Sample	Sex	Limits of Error from True Potency 95 Times in a 100	
		When Per Cent Responses are 20 and 80	When Per Cent Response is 50
5	M or F	74–136	76–131
10	M or F	81–124	83–121
15	M or F	84–119	86–117
20	M or F	86–117	88–114
25	M or F	87–115	89–113
30	M or F	89–113	89–112
35	M or F	89–112	90–111
40	M or F	89–112	90–110

The equation for the composite regression line for digitalis used in calculations of the above table is $Y = 4.98 + 13.1 (X - 1.63)$.

Note: In computing this equation all doses were multiplied by 10^8.

Table VII.—Limits of Error Computed for One-Dose Method, Ouabain Curve

No. of Frogs Used on Standard and Sample	Sex	Limits of Error from True Potency 95 times in a 100	
		When Per Cent Responses are 20 and 80	When Per Cent Response is 50
5	M or F	83–121	85–118
10	M or F	87–114	89–113
15	M or F	90–112	91–110
20	M or F	91–110	92–109
25	M or F	92–109	93–108
30	M or F	93–108	93–107
35	M or F	93–108	94–107
40	M or F	94–107	94–106

The equation for the composite regression line for ouabain used in calculations of the above table is $Y = 5.12 + 21.2 (X - 1.42)$.

Note: In computing this equation all doses were multiplied by 10^8.

and ouabain by a one-dose method. The figures given in these tables were computed from Irwin's equation 39 (4) in which the error of the slope of the standard curve is omitted. The error of the slopes for both composite curves is very small and may be neglected in comparison with the errors from other sources. The importance of the slope of the curve, the number of animals and the per cent response may be readily estimated from the tables.

DISCUSSION

The agreement between the slopes of the individual regression lines estimated by equation 20*a* (7), when considered in conjunction with the small differences in results of the two methods, Tables IV and V, is good evidence that one dose each of the standard and sample are sufficient for a test, and that the results may be calculated by curve numbers obtained from the standard Curve *D*.

The ouabain curve (Curve *O*) may be used in a similar way for the assay of strophanthus preparations, but, since Curve *D* and Curve *O* have different slopes, it would appear that the latter should not be used in calculating the results of digitalis assays. However, it is an interesting fact that, in 84 per cent of the assays, the differences from the three-dose method due to the use of this curve in calculation of results were 10 per cent or less. In those assays in which the variation was greater some of the moralities fell outside of the range 20 to 80 per cent.

An important reason advanced for the use of a three-dose method is the determination of significant changes in the slope of the dosage-response curve which might occur from time to time (2, 7), and it is recommended that the method be used in

routine assays for this purpose. Routine assays are necessarily carried out on limited numbers of animals, and even under ideal conditions, where the responses obtained are most effective for the calculations, the differences in slope which can be detected are relatively large. This may be shown by reference to the figures given in Table III, in which twelve separate determinations of the slope of the regression line for ouabain are reported. From seventy-five to one hundred and twenty frogs were used in each of the experiments and the slopes varied from 13.5 to 32.7, yet no *significant* difference could be shown in these twelve determinations. Chi-square for *b* was determined by Bliss' equation 20*a* (7) and also for each individual test in comparison with the composite line by his equation 20 (7).

In most cases it would appear, therefore, that relatively large differences in slope cannot be shown to have significance when the determinations are carried out in routine assays using the restricted numbers of animals which can be economically employed for this purpose. The evidence in Table III indicates that changes which have occurred in the case of ouabain were not large enough to be detected. When the slopes of the digitalis curve shown in Tables I and II were compared, no significant differences could be proven by equation 20*a* (7) and only three lacked agreement with the mean when equation 20 (7) was used. What appears to be of greater importance for the purpose of routine tests is the small variation in the results of assays when computed by the two methods, Tables IV and V. It appears, therefore, that a one-dose assay based on a standard curve will give results that are quite satisfactory in testing market samples of digitalis, and the method is to be preferred from the standpoint of simplicity.

Although the extent of the variability in the sensitivity of frogs to ouabain and digitalis is not the same, from a practical standpoint either the digitalis or ouabain curve may be used, providing the mortalities fall between 25 and 75 per cent. This observation has been emphasized in a recent paper by Chapman (10).

SUMMARY

1. Data for the construction of composite dosage-response curves for digitalis (*Digitalis purpureum*) and ouabain (*Strophanthus gratus*) are presented.

2. The composite curve for digitalis (*Digitalis purpureum*) has been found to differ significantly from the composite curve for ouabain (*Strophanthus gratus*).

3. A comparison of methods shows good agreement between a three-dose method and a one-dose method of assay for digitalis.

4. The maximum errors of the one-dose methods of assay for digitalis and also for ouabain are given.

5. A one-dose method is recommended for routine assays of digitalis and strophanthus preparations.

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Book Review

Handbook of Chemical Microscopy. Volume II. Chemical Methods and Inorganic Qualitative Analysis. EMILE MONNIN CHAMOT and CLYDE WALTER MASON. 2nd Edition. 438 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York, N. Y., 1940. Price, $5.00.

The second edition of Volume II of this handbook describes the essential manipulative methods employed in chemical microscopy and presents a compilation of the most dependable tests for the inorganic cations and anions. The references to original sources of information are unusually complete. This is believed to be the best and most complete authority in the field of microscopical qualitative analysis.—A. G. D.

The Fatty Acids of Carnauba Wax*,†

By Roy A. Bowers and A. H. Uhl††

Although the Carnauba palm (*Copernicia cerifera* Martius, Fam. *Palmæ*) was first described by Piso (1) in 1648 and further described by Raii (2) in 1688, Arruda (3) who published his description of the plant in 1810 is usually credited with its discovery. The leaves of the Carnauba palm yield carnauba wax, a valuable product which plays an important role in industry (4) and which is beginning to find its way into the art of pharmacy (5). Carnauba wax was discovered in the beginning of the eighteenth century by de Macedo (6). Steinle (7) has described the plant and wax in respect to its source and method of production.

Brande (8), in 1811, published the first experimental work on carnauba wax which consisted of a preliminary physical and chemical investigation. The work of Sturche (9), in 1884, with the exception of a few scattered reports (10, 11, 12, 13, 14, 15), has served as the only source of knowledge of the composition of the wax. Previous work suggested the following composition: *n*-heptacosane, all of the normal straight chain fatty acids containing an even number of carbon atoms, of from 24 to 30, a hydroxy acid with 24 carbon atoms, normal primary alcohols with an even number of carbon atoms of from 26 to 34, and a dihydric alcohol with 24 carbon atoms.

In the isolation, purification and identification of natural products the goal of the investigator must be that of obtaining the compounds in the condition in which they existed in the original state. However, investigations upon fats and waxes, with the exception of the recent work of Boemer and others (16, 17), have proceeded according to the time-honored method of the separation of the wax or fat into its saponifiable and non-saponifiable fractions. Of course, this does not elucidate the exact structure of the original naturally occurring substance. Since no methods of separating the constituents of waxes without a chemical change are known at this time, the conventional procedure was used in this work. Thus this investigation began with the saponification of the wax with alcoholic potassium hydroxide, removal of the alcohol and resolving the whole into soaps and non-soap portions. This separation was accomplished by the preferential solubility of the non-soap portion in Skelly-solve B and ethylene dichloride.

The isolation of unitary substances from natural fatty acids has been shown to be very difficult. Until about the last decade the general method of separating fatty acids was based on fractional crystallization. The multiple crystallization method employed by de Visser, in 1898 (18), was shown to be incapable of yielding a pure compound (19, 20).

Generally, the separation of homologs of fatty acids is best attained by fractional distillation of either their methyl or ethyl esters at low pressures through an efficient column (21). Longenecker (22) has prepared a review of the use of fatty acid ester distillation methods in fat analysis. He states that as early as 1877 Krafft (23) attempted the distillation of castor oil but succeeded only in cracking the oil. Bull (24) contributed materially to the problem of ester distillation in 1906. The introduction into the distillation system of vacuum pumps capable of continuously evacuating laboratory distillation apparatus to 1 mm. or less of mercury and the evolution of entirely new types of fractional distillation equipment have caused a rapid advance in ester distillation methods. Single turn glass helices (25) were shown to be particularly desirable for fatty acid ester distillations. Since 1934, Longenecker and others (26, 27, 28, 29, 30, 31) have used systems resulting from the development of theoretical knowledge of the fundamental principles involved in fractional distillations.

Schuette and Vogel (32) have shown that from the solidification points of a number of binary mixtures of fatty acids of known com-

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position one can plot a curve that is definite for every system of two acids. If one has a mixture of maximum resolution and if the mean molecular weight and solidification point are known, it is possible to calculate the composition of this mixture by means of the above curves. With this in mind a systematic rational fractionation of the methyl esters was made by means of ester fractional distillation and repeated recrystallization.

Preliminary experiments on the distillation of the methyl esters of the acids of carnauba wax showed that it was very difficult to fractionate the material into definite fractions. Francis *et al.* (33), in an attempt to obtain pure arachidic acid from a preparation obtained from peanut oil, made a systematic fractional distillation of its ethyl ester and finally obtained several fractions. From the corresponding acids which were repeatedly recrystallized they obtained X-ray data. No pure individual substances were obtained. Francis (34) reported that a complete separation of fatty acids by ester distillation methods was not possible. With this in mind an attempt was made to make a systematic fractional distillation to obtain fractions most likely to contain only two acids so that Schuette and Vogel's curves could be utilized.

Preliminary experiments also showed that about half of the methyl esters of the acids of carnauba wax would not distil without decomposition. Thus, the work that was done on the acids was solely that of the distillable portion. The following is an outline of the method used:

1. Saponification of the crude wax.
2. Separation of the soap from the non-soap portion.
3. Preparation of the methyl esters from the acids obtained from the soaps.
4. Distillation of the crude methyl esters into distillable and non-distillable fractions.
5. Fractionation of the distillable portion into like-boiling fractions.
6. Division into ten-degree distilling fractions.
7. Division into five-degree distilling fractions.
8. Saponification of the methyl esters into the corresponding acids.
9. Repeated recrystallization, determinations of neutral equivalents and solidification points until definite results were obtained from Schuette and Vogel's curves.

EXPERIMENTAL

The Apparatus.—Figure 1 represents the soda-lime, sulfuric acid and freezing traps. A mixture of powdered dry ice and acetone was used as the freezing medium.

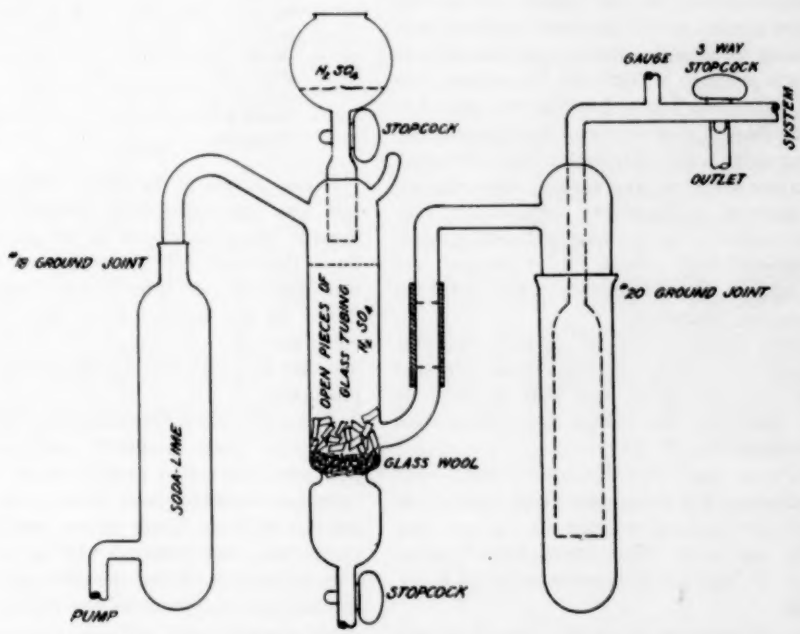

Fig. 1.—Soda Lime, Sulfuric Acid, Freezing Traps.

Figure 2 shows the combination column and receiver (35). The packing in the column is a single turn glass helix. The distillate was collected in an 8-inch test-tube and the opening closed with a rubber stopper. The column was heated with an outside heating similar to those used with a Widmer column.

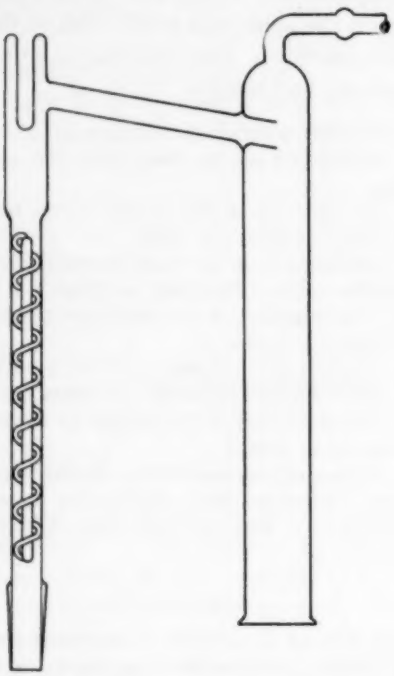

Fig. 2.—Combination Column and Receiver.

The system was evacuated over night before distillation was started. A Cenco-hyvac pump was used for evacuation and a modified McLeod gage for measuring pressures. Before any distillation was started a pressure of 0.01 mm. of mercury was obtained. Whenever a new fraction was placed in the distilling flask it was necessary to evacuate the system over night before distillation was continued. When fractions were cut the vacuum was released up to the point of the three-way stopcock, the rubber stopper removed, the fractions replaced by a new tube, restoppered with a fresh rubber stopper and evacuated again. About five minutes was necessary for the foregoing operation.

Solidification Points.—The method of determining the solidification temperature of fractions of methyl esters and free fatty acids was that of Schuette and Vogel (36). In the tables the solidification point is designated as S. Pt.

Melting Points and Resolidification Points.—For the determination of melting points and resolidification points the general method of Francis and Collins (39) was used. The melting point is designated as M. Pt. and resolidification point as R. Pt. in the tables.

Titrimetric Determination of the Mean Molecular Weights of the Acid Fractions.—The mean molecular weights of the acid fractions of carnauba wax were obtained through their neutralization equivalents. Seven-hundredths-normal, aldehyde-free alcoholic potassium hydroxide was used. This was standardized against potassium acid phthalate under exactly the same conditions as those under which the actual neutralization equivalents were taken. Sample weight of one-tenth gram was used and determinations carried out until checks of plus or minus two units were obtained. The determination was executed with a 250-cc. Erlenmeyer flask and a 10-cc. burette graduated to 0.05 cc. Alcohol (95 per cent) brought to the light pink of phenolphthalein with fiftieth-normal potassium hydroxide at 50 degrees was used as the solvent. The determination was as follows: The sample was dissolved in exactly 25 cc. of neutral alcohol with the aid of heat. The titration was carried out at 50–55°, adding the alkali slowly with continuous shaking, with phenolphthalein as indicator (3 drops of a 1 per cent solution in water). When five-tenths of a cc. from the end-point was reached, 30 cc. of distilled water was slowly added. The first appearance of a permanent light pink was taken as the end-point. The neutralization equivalent was calculated from the following formula:

$$\text{Neut. Equiv.} = \frac{\text{Weight of substance} \times 1000}{\text{No. of cc. } N \text{ alkali used}}$$

Determination of Constants.—The chemical and physical constants of the wax were determined according to the Pharmacopœial methods for Cera Flava (37).

Table I.—Chemical and Physical Constants of Carnauba Wax

M. pt.	76–78°
Sp. gr.	0.9897
Sap. no.	68.5
Acid no.	5.6
Ester no.	62.9
Iodine no.	10.5
Total ash	1.15 per cent
Moisture	4.8 per cent

Saponification of the Wax.—The crude powdered wax was saponified with alcoholic potassium hydroxide using an excess of 20 per cent of alkali. Four thousand one hundred and fifty grams of wax were divided into five portions and each refluxed for ten hours. After saponification the alcohol was removed completely at 17 mm. pressure. The dry material was removed from the flasks and powdered.

Extraction of the Non-Soap Portion from the Soaps.—Two to three hundred gram portions of the powdered saponified product were extracted with ethylene dichloride and Skelly-solve B. The extraction with the latter solvent was continued until completion and followed with acetone extraction. This extraction yielded the non-soaps as the soluble fraction and the soaps as the residue. The results of the saponification and separation of the soaps and non-soaps are herewith given:

Table II.—Proportion of Soaps and Non-Soaps

Amount of wax in grams	4150
Amount of soaps in grams	1300[a]
Amount of non-soaps in grams	2200

[a] A portion of soaps was lost due to an accident.

Preparation of the Free Fatty Acids.—The free fatty acids were prepared from the soaps by heating 200-Gm. portions with one liter of a 10 per cent aqueous solution of sulfuric acid at 85° and stirring for fifteen to thirty minutes. The conversion was indicated by the melting of the product. After cooling, the cake of fatty acids was removed and washed with hot water until free from mineral acid. The solid cake of acids was taken up in Skelly-solve B, water removed in a separatory funnel, filtered, the filtrate cooled and the deposited material collected. Nine hundred grams of wax acids melting at 68–72° were obtained.

For further and final purification of the acids, ether solutions at room temperature were prepared and the dissolved acids precipitated with a 10 per cent solution of sodium hydroxide. The sodium soaps were extracted with ether and Skelly-solve B. The acids were recovered as above and there resulted 850 Gm. of pure fatty acids.

Preparation of the Methyl Esters.—Seven hundred grams of acids were divided into three portions and esterified by refluxing with absolute methyl alcohol saturated with dry hydrogen chloride for 24 hours. The esters were recovered from the reaction mixture by removing the methanol, mixing the residue with a large volume of water, and alkalinizing with a dilute sodium bicarbonate and sodium carbonate solution. The mixture was heated to the melting point of the esters and stirred for a half an hour before cooling. The cake of crude esters was removed and extracted with Skelly-solve B. The Skelly-solve solution was refluxed with decolorizing charcoal, filtered, cooled in an ice-salt mixture and the precipitated esters collected on a Büchner funnel. There resulted 420 Gm. of crude esters melting at 68–69°.

Distillation of the Crude Methyl Esters.—The methyl esters were divided into four parts and each distilled through a short distilling head. The material was distilled from a 250-cc. flask packed to half is capacity with glass wool. The distillation was carried out under the following conditions: Distilling temperature of 150 to 220 degrees, a Wood's metal bath heated with a free flame at temperatures of 219 to 350 degrees, pressure of about 0.03 mm. of mercury. The distillation was stopped when the pressure began to rise steadily, indicating decomposition. A yield of 205 Gm. of distillate was obtained. The distilled esters were almost white in color, waxy in appearance and had a melting point of 63.5°. The corresponding acids had a carbon content of 22 to 26 carbon atoms as shown by the saponification equivalents of the esters.

Fractional Distillation of the Esters into Smaller Like-Boiling Fractions.—Before the material representing a mixture of methyl esters of fatty acids of high carbon content could be fractionated in a rational manner, the whole had to be reduced into smaller like-boiling mixtures. The esters were divided into two portions and each handled in like manner. The distillation was carried out in a 250-cc. flask packed to half its capacity with glass wool and connected to the column described in Fig. 2. Fractions were cut at the most uniform distilling points; however, there was constant variation in distilling temperatures. A Wood's metal bath served as a source of heat.

Table III gives the data for the five fractions collected:

Fractionation into Ten-Degree Distilling Range Fractions.—Each of the five fractions obtained in the foregoing fractionation was carefully separated into fractions distilling within 10-degree ranges. The fractionation started with Fraction 1 distilled from a 100-cc. flask packed to half its capacity with glass wool. When this fraction had been fractionated, Fraction 2 was added to the same flask and this treated in the same manner. This procedure was continued until all of the fractions had been separated into those of ten-degree distilling ranges. A bath containing Primex (hydrogenated vegetable fat) electrically heated with a coil of nichrome wire was used for the lower temperatures and a Wood's metal bath for the higher temperatures.

Table IV gives a summary of the results of the fractionation:

Fractions 1 and 2 were almost liquid and yellowish in color and so small in amount they were set aside. Fraction 3 was set aside as a finished fraction to be included among the five-degree range fractions. Fraction 4 was set aside for the same reason as Fractions 1 and 2.

Separation into Five-Degree Distilling Range Fractions.—The ten-degree fractions were fractionated into their corresponding five-degree fractions. One fraction at a time was fractionated in the same manner as was done in the preparation of the ten-degree fractions. See Table V.

Table III.—Constants of Five Fractional Distillates

Fraction	Wt. in Gm.	M. Pt., Degrees	S. Pt., Degrees	R. Pt., Degrees	B. Pt. Range, Degrees	Av. Pressure, Mm.
1	17.2	41.2	41.4	40.8	139–143	0.02
2	39.1	48.5	49.0	48.4	145–152	0.025
3	30.3	56.4	56.7	55.8	160–172	0.025
4	55.8	58.0	58.2	57.4	170–180	0.03
5	42.2	64.9	65.1	63.8	180–195	0.03

Table IV.—Constants of Refractionated Distillates

Fraction	Wt. in Gm.	M. Pt., Degrees	S. Pt., Degrees	R. Pt., Degrees	B. Pt. Range, Degrees	Av. Pressure, Mm.
1	0.55	..	...	...	95	0.012
2	0.94	..	...	..	95–105	0.02
3	2.51	27.4	27.3	27.7	105–115	0.02
4	1.16	37.9	...	38.2	115–125	0.025
5	27.29	41.6	42.10	41.8	125–135	0.025
6	8.79	46.8	47.95	47.5	135–145	0.022
7	83.32	56.9	57.23	57.2	145–155	0.022
8	11.60	60.4	61.10	60.5	155–165	0.03
9	44.63	62.2	63.90	64.4	Over 165	0.035

Table V.—Constants of Refractionated Distillates of Ten-Degree Fractions

Fraction	Wt. in Gm.	M. Pt., Degrees	S. Pt., Degrees	R. Pt., Degrees	B. Pt. Range, Degrees	Av. Pressure, Mm.
1[a]	2.51	27.4	27.30	27.7	105–115	0.012
2	6.40	40.2	40.10	39.8	115–120	0.02
3	9.24	42.2	42.70	42.1	120–125	0.02
4	2.14	44.9	44.82	45.0	130–135	0.02
5	6.73	50.0	49.96	49.8	140–145	0.025
6	9.01	50.7	51.69	51.1	145–150	0.025
7	69.70	54.4	56.20	54.4	150–155	0.03
8	5.15	57.1	58.16	57.3	160–165	0.04
9	1.48	58.6	58.28	58.9	170–175	0.035
10	16.71	61.4	62.63	62.2	185–190	0.04
11	28.21	63.0	65.17	64.0	Over 190	0.04

[a] Fraction 1 is Fraction 3 carried over from the ten-degree range fractions.

This concluded the fractionations.

Saponification of the Methyl Esters to the Corresponding Acids.—The recovery of the fatty acids from their methyl esters was accomplished by saponification with alcoholic potassium hydroxide. Together with a 20 per cent excess of alkali and enough alcohol to keep the material in solution, the mixture was refluxed for four hours. The alcohol was removed completely and the soaps extracted with Skelly-solve B, followed by ether extractions. The acids were liberated from the soaps in the same manner as before. Skelly-solve B solutions of the free acids at room temperature were shaken several times with anhydrous sodium sulfate and filtered. The solutions were concentrated to a small volume and decolorized with animal charcoal. The acids were obtained by freezing out in an ice-salt bath and dried in a vacuum oven at 48 degrees for eight hours at a pressure of 5 mm.

Table VI is a tabulation of some of the physical constants of the fatty acids obtained from the corresponding fractions of methyl esters:

Table VI.—Physical Constants of Fatty Acids Obtained from Methyl Ester Fractions

Fraction	Wt. in Gm.	M. Pt., Degrees	S. Pt., Degrees	R. Pt., Degrees	Mol. Wt.
1	1.60	60.2	60.78	60.2	294
2	3.75	69.0	69.32	69.2	291
3	6.76	71.4	71.46	71.4	298
4	1.11	66.1	68.01	67.0	320
5	5.36	72.8	74.51	73.7	330
6	5.51	71.3	71.46	71.2	338
7	58.70	78.6	78.78	78.2	349
8	3.45	76.1	77.51	77.2	375
9	0.84	77.4	77.18	77.9	388
10	11.86	80.2	80.92	79.4	399
11	24.95	84.0	85.62	84.6	424

After application of the above data to the proper binary mixture curves of Schuette and Vogel, it was seen that the fractions were not binary mixtures. Thus, recrystallization procedure was the next step. Each fraction was recrystallized twice from Skelly-solve B and twice from acetone in alternate order. One hundred cc. of hot solvent were used per Gm. of fraction; the solutions were allowed to cool for one hour in an ice chest at 12°, after which the deposited material was collected on a filter with the aid of suction and dried in a vacuum oven at 48° for 8 hours at a pressure of 5 mm.

The fractions obtained still did not give favorable results. However, Fractions 2 and 3 became practically the same in composition, as did Fractions 8 and 9. Thus, these fractions were combined, respectively. Each fraction was carried through the same recrystallization process as above. The solvent of the mother liquors was removed and the residues collected. The combined residues of Fraction 2 were combined and included in the general scheme as Fraction 2*a*.

Table VII is a tabulation of the constants of the fractions at this point:

Table VII.—Constants of Fractions Obtained by Crystallization

Fraction	Wt. in Gm.	S. Pt., Degrees	Mol. Wt.
1	1.60	60.78	294
2*a*	0.75	65.66	308
2, 3	4.35	73.38	311
4	0.40	71.46	320
5	2.75	75.12	346
6	3.26	73.90	353
7	...	80.62	363
8, 9	1.53	78.58	391
10	7.60	81.95	410
11	18.65	85.07	422

The composition of the fractions was determined by the use of solidification point curves of binary fatty acid mixtures of Schuette and Vogel (39).[1] These are used as follows:

From the observed mean molecular weight the proper curve is selected. The position of the solidification point of a fraction on the curve is noted. Two such positions are usually found. However, the observed mean molecular weight indicates which of these two positions is the correct one. If there is no coördination between the observed and calculated mean molecular weights, it may be assumed that the fraction is not a binary mixture. In this event further recrystallizations are necessary.

SUMMARY

The following is a summary of the conclusions drawn from the data obtained by application of each fraction to their respective curves:

Fraction 1.—The observed mean molecular weight placed this fraction in the C_{18}–C_{20} binary system. However, the solidification point was far below the range of this curve. The solidification point of its methyl ester was very close to that of palmitic acid but the mean molecular weight did not coincide. Since this fraction was small no further work was advisable.

Fraction 2a.—By application of the data for this mixture to the curve it was found that this fraction was composed of 31.5 mol. per cent of C_{18} and 68.5 mol per cent of C_{20} acids. The observed mean molecular weight and the calculated, 308 and 303.7, were in fairly good agreement.

Fractions 2 and 3.—This fraction had a composition of 3 mol per cent C_{18} and 97 mol per cent C_{20} acids. The observed and calculated molecular weights of 311 and 311.5 were in good accord.

Fraction 5.—This fraction was a mixture of 86.7 mol per cent of C_{22} and 13.3 mol per cent C_{24} acids. The observed molecular weight was 346 and the calculated 343.3.

Fraction 6.—This was a mixture of 67 mol per cent of C_{22} and 33 mol per cent of C_{24} acids. The observed molecular weight was 353 and the calculated 349.9.

Fraction 7.—The composition of this fraction was 14.5 mol per cent of C_{22} and 85.5 mol per cent of C_{24} acids. Theory for the molecular weight was 364.5, found 363.

Fractions 8 and 9.—By interpolation this fraction was 44 mol per cent C_{24} and 46 mol per cent of C_{26} acids. Theory for the molecular weight was 384.3, found 319.

Fractions 10 and 11.—Although curves were not available for acids of molecular weights in this group and since interpolation was not reliable at this point, C_{26}, C_{28} and possible C_{30} acids were indicated. Although the mean molecular weight of Fraction 11 was close to C_{28} acid, the solidification point was five degrees low. Many recrystallizations did not raise either the molecular weight or the solidification point of either of these two fractions. The molecular weight of both fractions might indicate only C_{26} and C_{28} acids but the depression of the solidification points of higher binary systems are not as great as those observed for these fractions. Thus there is good evidence of C_{30} being present and the presence of C_{26} and C_{28} acids can not be doubted.

Although there seems to be no question concerning the conclusions drawn from the application of data to the solidification point curves, one can employ a further medium by which the curves themselves will verify their own accuracy.

These solidification point curves have the form of a "W" (40). With increasing molecular weight the break in the center becomes flatter. For example, the C_{22}–C_{24} acid system has a plateau involving a composition of 30 to 70 per cent of C_{22} acid. Obviously, if the solidification point falls upon the plateau, it is impossible to calculate the composition of the mixture. Greatest accuracy can be obtained when the solidification point falls upon the most perpendicular portion of the curve.

Since the solidification points are very definite and the shape of the curve is also definite, a change in composition of any mixture in a definite system can be detected by a change in the solidification point. This change can be produced by the admixture of a definite amount of the proper pure synthetic acid. From this one can

[1] The solidification points of a number of binary mixtures of known composition are determined. From these a curve is plotted with the solidification points as the ordinate and composition expressed in mol. per cents as the abscissa.

compare the solidification points of the new mixture with that calculated for it. If the two points coincide, the original composition could be none other than what it was indicated to be in the first instance.

Fractions 2*a* and 7, inclusive, were examined by the admixture of the proper pure synthetic acid and the observed and calculated solidification points compared. The results in each case proved the initial conclusions to be correct. Hence, the presence of all of the acids from C_{18} to C_{28} and a good indication for C_{30} has been verified.

The plausibility of the existence of these acids mentioned above can also be seen in the table given below.

Table VIII.—Composition of Acid Fractions

Fraction	Composition
2*a*	31.5 mol per cent C_{18}; 68.5 mol. per cent C_{20}
2 and 3	3.0 mol per cent C_{18}; 97.0 mol per cent C_{20}
4	75.0 mol per cent C_{20}; 25.0 mol per cent C_{20}
5	86.7 mol per cent C_{22}; 13.3 mol per cent C_{24}
6	67.0 mol per cent C_{22}; 33.0 mol per cent C_{24}
7	14.5 mol per cent C_{22}; 85.5 mol per cent C_{24}
8 and 9	44.0 mol per cent C_{24}; 56.0 mol per cent C_{26}
10	Definitely C_{26} and C_{28} present
11	Definitely C_{28} and C_{28} present and possibly C_{30}

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Book Review

Chemical Publications, by M. G. MELLON. 2nd Edition. 284 pages. International Chemical Series. McGraw-Hill Book Co., Inc., New York, N. Y., 1940. Price, $2.75.

This is the second edition of the book by this title, the first edition of which appeared in 1928. The first chapter is given over to the introduction and general outline. This is followed by four chapters which deal with the original sources of published chemical information. The first subdivision of this class of material discussed is periodicals, the second is institutional publications. The next portion of the book covers secondary sources and includes a thorough treatment of index and review serials and abstract journals. The technique of making searches in the chemical literature is next considered and as a conclusion nineteen library problems are presented. —A. G. D.

The Effect of Some Adsorbents, Precipitants and Oxidants upon the Resin of Rhus Toxicodendron

*By Ole Gisvold**

It is common practice of the layman to treat ivy poisoning with the fresh juice of certain plants, more especially that of *Impatiens biflora*. The active substance present in *Rhus Toxicodendron* is phenolic in character. If the plant juices in question are actually effective *in vivo*, it seemed desirable to study their effect *in vitro*. All the experimental work presented in this paper is qualitative in character.

EXPERIMENTAL

The active principle of *Rhus toxicodendron* was prepared in the following manner. The petioles of the leaves were cut with a sharp instrument and the milky exudate was absorbed by small pieces of filter paper which in turn were immediately placed in alcohol to prevent a darkening of the latex. The alcoholic extract was used for the subsequent experimental work. This alcoholic solution gave a deep purple color with ferric chloride and color tests were used to trace the course of the qualitative tests. A portion of the extract was evaporated to dryness and the residue extracted with petroleum ether which removed an oily, extremely active substance.

The juice of *Impatiens biflora* gave a blue color with guaiac, a deep purple with α-naphthol and a positive deep red-brown precipitate (purpurogallin) with pyrogallol and hydrogen peroxide. The possible presence of an oxidase and a peroxidase was thus indicated. A hydro alcoholic solution of the active ivy principle was mixed with the fresh juice of *Impatiens biflora* and allowed to stand. It was tested from time to time with ferric chloride. A diminution in the intensity of the purple color obtained with ferric chloride was observed which persisted even after standing over night. If the active ivy principle was in sufficiently dilute concentration when mixed with the juice in question, a negative test with ferric chloride was obtained upon allowing the mixture to stand for several hours. It cannot be concluded from the above scanty test work that the active phenol present in *Rhus toxicodendron* can be oxidized by the oxidases present in the juice of *Impatiens biflora*. Perhaps in sufficiently dilute enough solutions, an oxidation may take place. Because the active ivy principle is quite insoluble in water, this line of investigation was temporarily discontinued to be supplanted by the subsequent chemical tests.

Precipitation with Lead Acetate.—A 2 to 3 per cent solution of lead acetate in 60 to 70 per cent alcohol completely precipitated the active ivy principle from a 70 per cent alcoholic extract of the ivy phenol. Upon standing, this precipitate soon turned a dirty blue. This precipitate was washed by repeatedly suspending it in 70 per cent alcohol and recovering the precipitate by means of a centrifuge. This precipitate when rubbed on the skin appeared to be fully as active as the original ivy principle. The same results were obtained with basic lead acetate.

Precipitation with Barium Hydroxide.—A 1 per cent solution of barium hydroxide in 60 to 70 per cent alcohol was added to a 70 per cent alcoholic extract of the ivy phenol. The mixture turned from a blue to a green color, which was immediately followed by a precipitate which was dark green. The supernatent liquid gave no color test with ferric chloride.

Precipitation with Aluminum Subacetate.—A 1 to 2 per cent solution of aluminum subacetate in 70 per cent alcohol gave no color change or precipitate. However, upon standing over night, a small amount of a flocculent green precipitate was obtained.

Precipitation with Aluminum Acetate.—No precipitate could be obtained with a 1 to 2 per cent solution of aluminum acetate in 70 per cent alcohol.

Precipitation with Magnesium Acetate.—A 1 to 2 per cent solution of magnesium acetate in 70 per cent alcohol gave no immediate precipitate with a 70 per cent alcoholic solution of the ivy phenol. However, after standing thirty minutes, the mixture darkened and a small amount of a precipitate appeared which settled after three hours. The mixture was still opaque.

Precipitation with Ferric Chloride.—Ferric chloride test solution was added to a 60 to 70 per cent alcoholic solution of the ivy phenol. A deep purple color was immediately obtained followed very quickly by a blue-black flocculent precipitate. This precipitate when rubbed on the skin appeared to be fully as active as the original ivy phenol.

Precipitation with Cupric Acetate—A 1 to 2 per cent solution of cupric acetate in 70 per cent alcohol was added to a 70 per cent alcoholic solution of the ivy phenol. A bluish black flocculent precipitate soon settled out. This precipitate was washed by repeatedly suspending it in 70 per cent alcohol and recovering it by means of a centrifuge. This precipitate when rubbed on the skin appeared to be fully as active as the original ivy phenol.

Adsorption by Aluminum Oxide.—A highly activated aluminum oxide was added to a 70 per cent alcoholic solution of the ivy phenol. The mixture was shaken for one minute and the mixture centrifuged. The supernatant liquid gave a positive test for ivy phenol and the intensity of the color indicated that little if any ivy phenol was adsorbed.

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Adsorption by Prepared Calamine.—The same technique described above was used with approximately the same results.

Adsorption by Magnesium Oxide.—The same technique described above was used. It was found that 0.1 Gm. of activated magnesium oxide completely absorbed the ivy phenol whereas 0.5 Gm. each of aluminum oxide or prepared calamine adsorbed little if any ivy phenol from an equal quantity of alcoholic solution. Glycerol up to 6 per cent does not inhibit the adsorption of ivy phenol on magnesium oxide. The magnesium oxide adsorbate of the ivy phenol was held in contact with the skin for twenty-four hours by means of gauze and tape. The results indicated that most if not all of the adsorbed ivy phenol became effective.

Oxidation with Alkaline Peroxide.—A 3 to 5 per cent alcoholic solution of hydrogen peroxide was added to a 70 per cent alcoholic 0.5 per cent sodium hydroxide solution of the ivy phenol. The solution immediately assumed a light pink color. The alkali was neutralized and the resulting mixture gave no color when tested with ferric chloride. The ivy phenol forms a water-soluble green salt with sodium hydroxide. It gradually darkens at the surface and upon exposure to air slowly assumes a pinkish color. Aqueous sodium carbonate does not dissolve the ivy phenol; however, this mixture upon standing assumes a pinkish color.

The author of this paper was the test subject. Alkaline peroxide was used which proved quite effective in the treatment of ivy poisoning. The alkali was sponged over the affected areas followed by the peroxide applied in the same manner. After a few minutes, the alkali was neutralized with a weak solution of acetic acid. Two or three such treatments, once daily, appeared quite sufficient to destroy the ivy phenol that was not too deep seated and prevented it from spreading. Where the lesions were quite deep, the treatment was somewhat painful, but of short duration.

SUMMARY

A preliminary qualitative study has been made upon the effect of some adsorbents, precipitants and oxidants upon the physiologically active phenols found in the milky exudate obtained from *Rhus Toxicodendron*. Calamine and aluminum oxide were found to be extremely poor adsorbents for these phenols. Magnesium oxide was very active as an adsorbent; however, the adsorbate of the ivy phenol when used as such was physiologically very active as a vesicant. Lead acetate and basic lead acetate were the best precipitants found; however, the precipitates were also very active. Oxidants such as ferric chloride and cupric acetate gave precipitates which were also very active. The only successful method studied of rendering the active phenols inactive, involved their oxidation with alkaline peroxide.

The Chemistry of Burbot Liver Oil—I*

By R. T. Lakey,† F. W. Mittelstadt‡ and M. G. deNavarre

The Burbot fish, a fresh water cod, is commonly found in all of our large inland lakes and streams. It is not usually used as food, but has some worth as fertilizer. The average weight of the Burbot is about 5 pounds. The liver is a good deal larger than that of any other fish in the same waters. One such liver used in experimental work weighed 900 Gm. The liver contains from 10% to 56% of oil, the exact amount probably depending somewhat on the age of the fish as well as the water from which it was taken.

Glow and Marlatt (1) have reported the antirachitic potency of Burbot Liver Oil (hereafter abbreviated B. L. O.) as eight times that of cod liver oil, and classed it as an excellent source of vitamin D.

Myers (2) treated fifty infants 1 to 2 months old over a period of 6 to 12 months, and reported the B. L. O. to be a good antirachitic agent having a vitamin D potency approximately 8 times that of cod liver oil.

Branion (3) reported that B. L. O. to be a good anti-rachitic agent and that its activity in treating xeropthalmia was greater than that of cod liver oil.

The above is all more or less recent work on B. L. O. vitamin potency; preliminary study on B. L. O. dates back to the work of McCollum and co-workers (4) who found vitamin D in the oil as early as 1922.

The chemistry of this oil, however, has not been given the attention it deserves for the

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* This project was sponsored by Maison G. deNavarre Associates, Consulting Chemists, Detroit, Michigan. The analytical investigation was done principally by F. Mittelstadt, Graduate Student, Wayne University College of Pharmacy.

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scientific literature shows only one recent report on the analysis of the oil, and some work by Lewkowitz in his Volume II, of an oil which he calls "Ling Fish Liver Oil," a type of European Burbot. A recent report by Nelson, Tolle and Jamieson (5) gives a partial analysis of the oil as well as a report of its vitamin activity.

Table I shows our findings on a standard lot of oil blended from several batches. Figures in the table are the average results of many tests. Due consideration was given to the oxidizability of the oil, and a layer of carbon dioxide protected the oil from the air whenever possible.

Table I

Specific gravity, 25° C.	0.9219
Refractive index, 20° C.	1.4776
Optical rotation[a]	−1.3°
Viscosity Saybolt, 27.8° C.	0.376
Viscosity Saybolt, 37.8° C.	0.258
Moisture and volatile matter[b]	0.12
Crismer turbidity	71.56° C.
Acid value	0.25
Saponification value	189
Iodine value (Hanus)	144.1
Unsaponifiable matter, %	1.14

[a] In 200-mm. tube, sodium light, 82° F.
[b] 3½ hours at 100–105° C. in atmosphere CO_2.

These figures are slightly at variance with those of Nelson, Tolle and Jamieson, and with those found in the New and Non-Official Remedies for B. L. O.

It has been claimed that therapeutic response to B. L. O. is superior to that of C. L. O. Accordingly, we will continue the investigation of the chemistry of B. L. O. in order to determine, if possible, the reason for this superior therapeutic usefulness. Our investigation is to be continued. Later papers will treat of the nature of the fatty acids and esters present in this oil.

SUMMARY

The two points of interest in the report of this investigation are: First, a more complete analysis of the constants of a blend of several batches of burbot liver oil than any that we have seen published heretofore: second, the results of our analysis differ somewhat from those reported by other workers.

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Papain as a Precipitant of Gums*

By George E. Éwe†

In the June 1930 issue of THIS JOURNAL, the writer reported on methylene blue as a precipitant of Irish moss as a contribution to the subject of known means of identifying and differentiating gums. The present note is concerned with the use of papain as a precipitant of gums.

EXPERIMENTAL

Upon attempting to incorporate powdered commercial papain into a fluid preparation containing Irish moss, flocculation of the latter was observed. When papain is added to a Irish moss decoction at room temperature, a ropy, gelatinous, insoluble mass separates out. This action is progressively lessened as the papain solution is progressively more highly alkalinized.

The papain used in these experiments was acid to litmus, methyl red and phenolphthalein, but only slightly so. The papain could be precipitated from aqueous solution by hydrochloric or sulfuric acids whereas the Irish moss decoction could not be precipitated by gross addition of either of these acids. Consequently the flocculating power of the papain does not appear to be a function of its gross acidity.

Flocculation of Irish moss decoction by papain solution is not prevented by adding sodium benzoate, sodium acetate or sodium borate to the papain solution. This is also true of magnesium sulfate, ammonium sulfate, sodium sulfate, sodium succinate, zinc sulfocarbolate and Rochelle salt. Sodium thiosulfate decreased the flocculating action of papain upon Irish moss decoction to some extent. Dibasic sodium phosphate, sodium chloride, potassium chloride, ammonium chloride and sodium glycerophosphate all greatly decrease the flocculating action. Neutral potassium tartrate dissolved in the papain solution reduces the flocculating action of the papain to a much greater extent than any of the above-mentioned substances and

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does not seriously affect the taste of the mixture when employed in a useful proportion, thus giving passable results for some pharmaceutical purposes.

Papain solution also flocculates "solutions" of Karaya gum, quince seed mucilage and sodium alginate, yielding stringy, gelatinous masses. It also causes a whitish, flaky precipitate in "solutions" of locust bean gum and agar-agar. It does not produce striking results when added to "solutions" of acacia, tragacanth, gelatin or sassafrass pith mucilage.

When proteolytic activation of the papain is assured by using hydrogen sulfide water for making its solutions, the same results are obtained.

While the flocculating action of papain on certain gums may prove useful in identifying and differentiating gums, variations due to processes to which the gums have been subjected must be taken into account in interpreting results obtained by the use of papain. For instance, the aqueous liquid, separated by settling, from a preparation made with sodium alginate gave a dense, milky, flaky precipitate instead of the stringy, gelatinous masses yielded by a freshly prepared "solution" of the same sodium alginate in water. Likewise, the aqueous liquid separated by settling, from one preparation made with Irish moss, gave the usual ropy, gelatinous, insoluble masses, while that from another preparation also made with Irish moss yielded dense, milky, gelatinous flakes.

It is also of interest to note that papaw juice can be used for coagulating rubber latex (1) in place of acetic acid, but whether this action is due to the natural acidity of the juice or to some other action of the juice upon gums is not stated in the article cited.

The cause of the flocculating action of papain upon some gums was not ascertained. Papaw juice contains, besides papain, a milk-curdling enzyme (2). Papain contains proteolytic enzymes (3, 4, 5). If the flocculating action reported in the present article is due to curdling enzyme action upon the protein or nitrogenous matters accompanying most gums, this action is selective and is like the action of most curdling enzymes in this respect.

When papain is previously heated at 95° C., it acts as a precipitant of proteins from bouillion (6) and this would seem to indicate that papain will precipitate certain proteins aside from any curdling enzyme action.

It has been observed by Racicot and Ferguson (7) that Irish moss is precipitated by proteins in an acid solution and, since commercial papain contains a number of proteins, its flocculating action upon irish moss may be a function of its protein content. However, the addition of acid is not required to bring this about.

Another possible explanation of the flocculating action of papain which has not been subjected to study as yet is that the action may be due to the mixing of colloids of opposite electric sign.

SUMMARY

Papain is herein reported as being a precipitant of certain gums.

The report of this action of papain is made as a contribution to the subject of means of identifying and differentiating gums.

The cause of this precipitating action is not established but possible explanations for this property of papain are discussed.

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Thiamin Chloride and Bismuth Tri-Iodide Complex

*By C. S. Leonard**

In the course of pharmacological studies, the insoluble complex formed from thiamin chloride and Dragendorff's reagent (a reaction cited by Naiman (1) as a qualitative test for thiamin) was prepared and analysed.

EXPERIMENTAL

The red, microcrystalline precipitate was made by adding Dragendorff's reagent (2) to a 10% solution of thiamin chloride hydrochloride (Merck[1]) in centrifuge tubes or bottles until precipitation was complete and a red-orange color appeared in the supernatant fluid. After removing the mother liquor by centrifugation, the complex was washed repeatedly by stirring up in water and centrifugation, until the washings were colorless. It was then filtered, washed once on the filter and dried *in vacuo* over anhydrous calcium chloride. The yield from 1 Gm. thiamin chloride hydrochloride was 5.15 Gm. Several batches were thus prepared.

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[1] Kindly supplied by Merck and Co., Rahway, N. J.

If analogous to the complexes of bismuth tri-iodide with various organic amines described by Kraut (3), one mole of amine halide or of base might be combined with one or two or more moles of bismuth tri-iodide. It was found that the formula $C_{12}H_{17}OSN_4Cl.HCl.2BiI_3$ gave calculated bismuth and nitrogen percentages closely approximating the analytical values. Formulas constructed with the amine base, or with potassium bismuth iodide, did not fit as well. The compound would appear quite analogous chemically to quinine iodobismuthate which is cited by François and Seguin (4) as having the formula $C_{20}H_{24}N_2O_2.2HI.2BiI_3$. If the precipitate of the quinine complex and its mother liquor were heated with excess hydrochloric acid, François and Blanc (5) obtained a crystalline compound with the formula $C_{20}H_{24}N_2O_2.HI.3BiI_3$. Variable composition of quinine iodobismuthates was noted by Vita and Bracaloni (6) according to the amount of washing of the precipitate. We have obtained precipitates of thiamin and bismuth tri-iodide which, when less thoroughly washed than the preparations cited as to analysis below, contained 21.0–21.1% bismuth. If the conditions of washing were fixed, however, the batches were quite uniform in composition.

The substance was insoluble in water. Like sodium iodobismuthite (Hanzlik *et al.*, 7) and quinine iodobismuthate (Picon, 8) the thiamin bismuth complex was found to be somewhat soluble in ethylene glycol or propylene glycol or glycerol. It was slightly soluble in acetone, insoluble in ethyl acetate.

For convenience in biological work, the compound was given the short name of bismothiamin. Pharmacological data on the toxicity and rate of mobilization of this preparation after intramuscular injection in animals will be published elsewhere.

Analysis. Calcd. for $C_{12}H_{17}OSN_4Cl.HCl.2BiI_3$: Bi, 27.6%; N, 3.69%.

Found: Bi (Leonard's (9) colorimetric method), 27.3%, 27.7%; N (micro-Kjeldahl[3]), 3.28%.

SUMMARY

The insoluble, red addition compound formed by the precipitation of thiamin chloride with Dragendorff's reagent was analysed as to content of bismuth and nitrogen, and was found to correspond to the formula: $C_{12}H_{17}OSN_4Cl.HCl.2BiI_3$, containing 27.6% bismuth. For biological work it is called bismothiamin. In view of its chemical analogy to quinine iodobismuthate, the pharmacological properties of bismothiamin are being studied.

[3] I am indebted to Professor C. E. Braun for the N analyses.

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A Pharmacognostic Study of Digitalis Ambigua

*By Barbara Jacobs**

INTRODUCTION

In view of the fact that various reliable sources have reported *Digitalis ambigua* to be as efficient therapeutically, as *Digitalis purpurea*, it seems timely to collect and organize the information available, and to complete the anatomical picture for this potentially useful species of the *Digitalis*.

In their whole state, the leaves of the various species of *Digitalis* are sufficiently distinctive to prevent confusion. In the powdered state, however, they are very difficult to distinguish. Although they are all similar with respect to the nature of their physiological action, they vary in strength, and it is therefore essential to establish characteristics so that acceptable species may be differentiated from undesirable species.

In this report I have confined myself strictly to the pharmacognosy of the subject. The chemistry, pharmacology and therapeutics involved are only cited to justify the value of the work.

All drawings have been made directly from fresh material obtained by request from the University of Minnesota College of Pharmacy.

* Prize winning paper; Kilmer award.

NOMENCLATURE

There have been so many names applied to this species of *Digitalis* that there is considerable confusion in recognizing them all as the same plant.

According to Maheu and Chartier (1) the following names have been applied:

Digitalis grandiflora All.
Digitalis grandiflora Rchb.
Digitalis ochroleuca Lindl.
Digitalis ochroleuca Jacq.
Digitalis ochroleuca Rchb.
Digitalis ambigua Murr.
Digitalis ambigua Lindl.
Digitalis ambigua Sturm.
Digitalis orientalis Mill.
Digitalis flava Georgi
Digitalis lutea Maja
Digitalis lutea Pole
Digitalis purpurascens Roth.
Pentstemon frutescens Lamb.

REVIEW OF LITERATURE

The first scientific article devoted to this species of digitalis appeared in the literature in 1888. Paschkis (2) in Germany determined the constituents and reported them as being identical to those found in *Digitalis purpurea*.

In 1893 Goldenberg (3) of France was experimenting with some unknown species, one of which proved to be *Digitalis ambigua*. He reported that it had the same type of action as *Digitalis purpurea*, but varied in energy of action.

The next report by Hans Benyschek (4) in 1900 was concerning this variance in energy. He claimed that the yield of proximate principles in the various species depended inversely on the amount of water storage, which in turn, varied with the locality and the time of year during which the plants were collected. In this he infers that actually there may be little difference in energy if all species were grown and collected under the same conditions.

Two years later Jaccard and Golaz (5) worked out in great detail the chemical composition of the four species best known in Switzerland and France, namely: *purpurea, lutea, lanata* and *ambigua*.

In 1904 Boudgest (6) described this plant under the name of *Digitalis grandiflora* as a yellow-flowered species of digitalis which was found abundantly in Switzerland and which possessed activity as a cardiac remedy as pronounced and equal to *Digitalis purpurea*. He claims that this has been demonstrated by previous investigation, and that there is no longer any ground for the rejection of the leaves of *Digitalis ambigua* as a medicinal agent.

Dr. James Burmann (7) completed in 1912 an assay conducted over a period of five years. Among other drugs it included fresh leaves from wild growing plants of *Digitalis ambigua* and *Digitalis purpurea* collected at flowering. They were assayed for digitoxin by the gravimetric method of Keller and the following results obtained.

	D. ambigua, %	*D. purpurea*, %
1907	0.134	0.078
1908	0.120	0.063
1909	0.067	0.033
1910	0.069	0.037
1911	0.148	0.070

In 1916 researches conducted in the Pharmacognostic Institute of the Vienna University (8) indicated that *Digitalis ambigua* was an acceptable substitute for *D. purpurea*.

A year later at the University of Minnesota, in this country, R. E. Morris (9) made a pharmacological comparison of twenty-three species and numerous varieties. He reported *D. ambigua* as equal to *D. purpurea*, and made the general statement that if care in use, standardization and accurate dosage were practiced that the species or variety was of little importance.

D. ambigua was not again mentioned in the literature for over ten years. The next article appeared in 1929 and concerned standardization. *D. ambigua* was cited as the only spontaneous species and very common in Italy. The determination of digitalin was carried out by the Keller-Ecolle method and showed 0.078% digitalin. Since the medicinal activity is not due entirely to this principle another assay by the Foke's method (modified) was conducted. Conclusions were that *D. ambigua*

was less active than *D. purpurea* in the ratio of 0.75 to 1 (10).

In 1934, Maheu and Chartier (1) of France did the only extensive pharmacographical work that has been done on this species. They worked out the morphologic and anatomic characters of whole leaves and powder. Their descriptions have been used in this paper as a guide during actual observations.

This same year, Hocking (11) gives us his viewpoint on the relative strength of the active principles. He opposes some of the earlier reports by stating that according to composition, fertilizer and treatment, the soil exercises a predominant influence on the actual strength of the active principles. He does agree, however, that the climate and frequency of cutting have great influence on both the yield and the strength.

About this time there was considerable experimenting with hybrids of *D. ambigua* and *D. purpurea*. Giant plants were bred but have not proven of any great value (12).

The last important reference to this species was in the "Quarterly Cumulative Index Medicus" for 1937, where it is definitely reported that *D. ambigua* has been used in medicine successfully.

HABITAT

Digitalis ambigua is indigenous to Europe and Western Asia. It is especially abundant in the woods and rocky mountain areas of Switzerland, France, Austria and Italy. It has been found to some extent anywhere across central Europe from Spain to Russia and western Siberia.

It has been naturalized in the United States and has for some years been successfully grown at the University of Minnesota.

Fig. 1.—*Digitalis ambigua*. Flowering and Fruiting Branch, Natural Size.

EXPERIMENTAL

Examination of the Entire Plant.—Digitalis ambigua is a perennial herb growing from 50 cm. to a meter in height. The stems are cylindrical, green, pubescent and sometimes ribbed. The leaves are numerous, simple, alternate, exstipulate and sessile or clasping.

The inflorescence is a simple raceme of yellowish campanulate flowers. The calyx is hairy, usually with five linear-lanceolate lobes which are recurved at their ends. The corolla is from three to four cm. long, and from one and one-half to two cm. wide. The petals are yellow and are reticulately veined with brown on the inside, pubescent on the outside. Long green bracts are present at the junctions of the pedicels and rachis.

The fruit is a two celled, ovoid, pubescent capsule with central placentation and many seeds. The seeds are minute, brown to reddish brown in color, and irregular in shape. Their outer surface appears

finely reticulate with a groove-like depression on the ventral side, and there is a distinct micropyle at the apex of the groove.

Physical Characteristics of Leaf.—The individual leaves are dark green in color and ovate-lanceolate in shape, with a closely toothed margin. The apex is acuminate, the base tapering into a flat wide petiole which clasps the stem. The upper surface is glabrous, shiny and nearly smooth in comparison with *D. purpurea*. The lower surface is somewhat pubescent, especially along the margin and over the veins. The venation is distinct, the veins being large, brown to purple in color, and not closely reticulate. They are convex toward the lower surface, and the vein-eyelet number is from 2 to 2.5.

The odor is characteristically pungent, the taste is bitter.

Fig. 2.—*Digitalis ambigua*. Leaves and Stem; Natural Size.

Microscopic Examination of a Transverse Section of a Leaf.—The upper epidermis is composed of clear cells which appear rectangular in cross section. The inner and radial walls are slightly concave and somewhat thickened, the outermost wall is convex and shows a cuticle. There are only occasional stomata on this surface, and a few hairs or hair bases from which the hairs have broken off. These hairs, both glandular and non-glandular, are described in detail under the powdered drug.

Immediately below the upper epidermis is a single layer of palisade chlorenchyma, continuous except in the region of the midrib where it is replaced by collenchyma cells thickened in their angles. The rest of the mesophyll region consists of large rectangular cells and numerous intercellular air spaces. Coursing through this region are numerous fibrovascular bundles of the open collateral type. The vascular tissue of the midrib consists of an arc of xylem made up of reticulate and porous tracheæ and islets of sieve tubes.

The lower epidermis is similar to the upper in this view except that stomata and hairs are more numerous.

Powder.—In dilute alcohol mount numerous epidermal fragments can be clearly seen in surface view. Those from the upper epidermis are composed of subhexagonal cells with slightly wavy thickened walls, and sometimes granulations similar to those of *D. lutea* but fewer and more regularly distributed. Stomates with three to four neighboring cells are seen occasionally. Sections from over the large veins are made up of regular hexagonal cells which have thick walls and a striated cuticle. The lower epidermis is formed of hexagonal cells the walls of which are thickened and seem to vary from wavy to rectilinear. They are granulated only slightly or not at all. Stomates similar to those of the upper epidermis are numerous.

There is considerable fibrovascular material from the large veins which consists mostly of lignified reticulate and porous tracheæ.

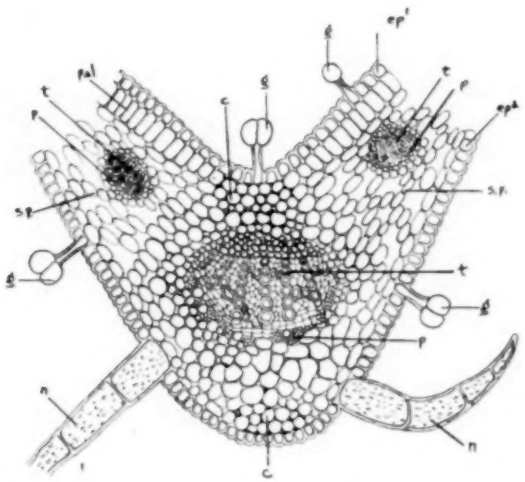

Fig. 3.—*Digitalis ambigua*. Transverse Section of Leaf: *ep*¹, upper epidermis; *ep*², lower epidermis; *pal*, palisade parenchyma; *s.p.*, spongy parenchyma; *t*, tracheæ; *p*, phloem; *c*, collenchyma; *n*, non-glandular hair; *g*, glandular hair.

There are both grandular and non-glandular trichomes present, but they are few in numbers as compared with powdered *D. purpurea*. The glandular hairs have an elongated one-celled stalk, and either a one- or a two-celled head. The non-glandular hairs are large and uniseriate up to four or five cells. They have thickened walls and numerous projections or nodules elongated in the direction of the length of the hair. These appear white

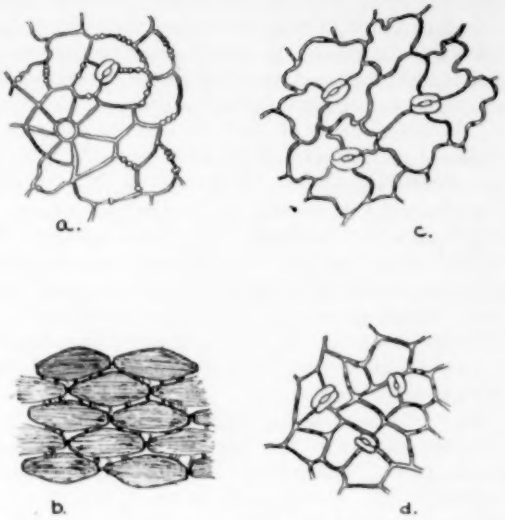

Fig. 4.—*Digitalis ambigua*. Epidermal Tissue: *a*. Upper epidermis showing granulated walls and hair base; *b*. Upper epidermis over large vein showing striations; *c*. Lower epidermis showing wavy walls and numerous stomata; *d*. Lower epidermis showing rectilinear walls and stomata.

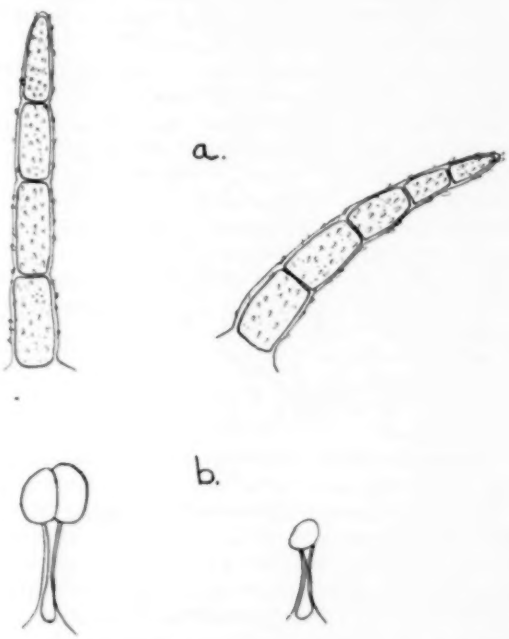

Fig. 5.—*Digitalis ambigua*. Hairs: *a*. Non-glandular, × 110; *b*. Glandular, × 250.

under high magnification and give the hair a warty appearance.

The most diagnostic and distinguishing characteristics of powdered *Digitalis ambigua* are the surface sections and the non-glandular trichomes as described above.

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The Examination of and Standards for Imported Drugs from 1790 to 1908*

By Lyman F. Kebler†

Following the presentation of my paper on establishing the Drug Laboratory, before this section, at the Atlanta meeting, a prominent member of this Association, reminded me of the low quality of some of the imported drugs for many years and the findings of Dr. Rusby, in particular, presented at a meeting of this Association abut the time the Drug Laboratory was organized, and asked why we did not start on the import work

* Presented to the Historical Section A. PH. A., Richmond meeting, 1940.

† Former Chief of the Drug Laboratory, Bureau of Chemistry, United States Department of Agriculture.

earlier? I explained to him as well as I could briefly that the work on drugs, at the ports was started as soon as feasible and funds were available. In order to make the information more readily available, I thought it might be of service to present a brief sketch on the examination of drugs and the standards therefor, at the ports of entry, covering a little more than a century. The examination of imported drugs and chemicals began with the tariff act of 1790 (1). This act provided, among other things, for the inspection and testing of wines and alcohol. Except for revenue purposes, tariff law standards are of little value. Later tariff acts gradually expanded the work, but adulterated, spurious and low grade drugs and chemicals kept coming into the country. Pharmaceutical chemists exposed and continued to expose, some of these shortcomings for years without any apparent improvements. The leading apothecaries and physicians became greatly disturbed. Improvements in drug standards and education came slowly in our country. The United States Pharmacopœia came in 1820 and several colleges of pharmacy were founded previous to the enactment of the 1848 drug and chemical import law.

THE 1848 DRUG AND CHEMICAL IMPORT LAW

This law was enacted largely through the combined efforts of the medical and pharmaceutical professions. The latter exposed some of the villainous adulterations and substitutions, memorialized Congress, and the former, in a memorial to Congress, pointed out the harm done by such medicines to both physicians and their patients. These unfortunate and even serious conditions appealed to members of Congress. Senator John A. Dix, of New York, in April 1848, introduced a bill on the subject. In the House a Committee was appointed to study the problems. Hearings were had. Some awful conditions were disclosed. Representative Thomas O. Edwards, a physician of Ohio and chairman of the above Committee, gave the subject close attention, particularly from the viewpoint of physicians. On June 2, 1848, he reported (2) for the Committee and submitted a bill to the House, which bill was speedily passed (3) and referred to the Senate. The Senate substituted the Dix Bill, which bill was somewhat amended, passed both branches of Congress and became a law June 26, 1848 (4). This law recognized as authorities the United States, the Edinburgh, London, French and German Pharmacopœias and Dispensatories. It must be said that this law was most liberal in the number of authorities recognized. All of these works conained good methods for determining the purity of many chemicals used as medicines but were defective in the matter of crude plant drugs and their derivatives.

The enforcement of this law was placed in the hands of the Secretary of the Treasury. On July 8, he issued instructions, directing that the drugs, chemicals and medicines, imported from the respective countries, whose pharmacopœias and dispensatories were recognized by the law, must comply with the standards contained therein and that all other drugs, chemicals and medicines, originating in other countries must comply with the United States Pharmacopœia and Dispensatories. This law has never been repealed.

The Secretary of the Treasury on October 10, 1848 appointed (5) Congressman Edwards to study and ascertain how well the law was enforced and working. The Doctor made a very favorable and exhaustive report (6).

Dr. M. J. Bailey, special examiner at the port of New York, reported (7) on the practical operation of the law June 9, 1849. This report in a measure supported the findings of Dr. Edwards but was less optimistic. Dr. Bailey pointed out that there are few physicians, even recent graduates of our best medical schools, versed in the practical detection of drug adulteration and that the majority of those who prepare and sell drugs are deficient therein. This is in a measure true even in our own time.

The ugly head of politics, in the matter of appointing drug examiners, soon intruded itself, and became an early disturbance. The best interests in the drug trade combatted this intrusion but lost heart in the end. Political preference and not ability obtained. In this connection the reader is referred to Curt P. Wimmer's "History of the College of Pharmacy of the City of New York," page 203 (1929). He certainly presents an assortment of troublesome elements then worrying the apothecaries.

LACK OF EFFICIENT STANDARDS FOR LAW ENFORCEMENT

A lack of efficient standards for some drugs for law enforcement purposes, soon became evident. This was true for all of the authorities recognized at the time. The pharmaceutical profession became greatly annoyed. Reliable standards must be had for medicines. The Trustees of the New York College of Pharmacy became so disturbed because of the lack of standards for some important drugs and the difficulties resulting therefrom, that they sent invitations to the then existing Schools of Pharmacy, to send delegates to a meeting called for October 15, 1851 (8). At this meeting Dr. Charles B. Guthrie was elected president. The belief prevailed that the lack of adequate standards could not be over-emphasized. Standards were proposed for a number of drugs, but unfortunately no methods for determining the several percentages

proposed, were provided. Why the job was not completed the records do not show. The lack of reliable standards harks back to the II or 1840 revision of the United States Pharmacopœia, a work which the revisors had no idea would become a legal standard.

These difficulties were pressing at the time, when the III or 1850 revision of the United States Pharmacopœia, there were two editions, was under way. Why the Committee of Revision did not, under the circumstances, provide better standards with methods of analysis is not clear. There certainly were some methods available at the time, to provide for some of the deficiencies. Under the cricumstances the port examiners were compelled either to utilize the existing methods, or devise some of their own, or let the proposed standards go by default. Under the conditions uniformity at the various ports was not possible. It is interesting to note that standards for some drugs were published in 1857 (9) by the Treasury Department, but again no methods were given. Virtually the same standards were published by the Treasury Department as late as 1884, but still no methods of analysis.

REPORT OF DRS. GUTHRIE AND BAILEY ON WORKINGS OF 1848 LAW

The Secretary of the Treasury commissioned Dr. Charles B. Guthrie to study the workings of the law. His general report (10) is full of the good things the law accomplished and was accomplishing, but there was still much to be done. He pointed out that the imperfect standards for crude drugs and medicines in works on pharmacy and materia medica made it necessary for each examiner to fix his own standard for such important drugs as opium, scammony and the cinchona barks, which did not make for uniformity. He pointed out that the publicity given the subject was working a revolution in the drug trade. Public opinion demands it. Said he: "A man is no longer considered competent to sell, dispense and deal out medicinal articles affecting the health, life and happiness of his fellow-beings, simply because he can calculate a percentage or make a profit. Physicians, professors of materia medica and teachers of practical pharmacy and chemistry are feeling it."

From a reading of this report one gets the impression that the detailed report, referred to, copy of which I have been unable to locate, might not make things look so favorable. The political discord in the matter of the drug examiners gives color to this idea.

Dr. M. J. Bailey's report made about the same time (11) presents quite a different picture, of conditions obtaining regarding drug importations. He calls attention to the large amounts of drugs rejected by him at the port of New York, since the enactment of the law. He alleged that he had rejected all told 610,000 pounds of adulterated, spurious drugs and drugs unfit for medicinal use. Among the rejections may be cited: 304,135 pounds of spurious Peruvian bark, 31,838 pounds of senna, 6864 pounds of iodine and 3720 pounds of potassium iodide. He observes that the best devised system of standards and tests, would be of little avail, unless the work is entrusted to a personnel having the requisite education, and being honest and competent.

DRUG ADULTERATION AND DEFECTIVE STANDARDS THE IMMEDIATE CAUSES FOR ORGANIZING THE AMERICAN PHARMACEUTICAL ASSOCIATION

Previous to the adjournment of the above assembled delegates, provisions were made for the President to send invitations to authorized bodies, to name delegates to a convention to meet the first Wednesday in October 1852, for the purpose of organizing a National Association, to meet annually. The call for such a convention, to meet in Philadelphia, October 6, 1852, was issued by the President, Dr. Guthrie. The delegates assembled in Convention, in accordance with the call (12). President Guthrie was unable to attend. This Convention resulted in the formation of the AMERICAN PHARMACEUTICAL ASSOCIATION. It may therefore be said that the defective standards and adulterations were the immediate causes for the formation of this ASSOCIATION in 1852.

One would naturally assume that with all of this agitation about defective standards and lack of methods of analysis, the voids would have been bridged to some extent at least, by the IV or 1860 revision of the United States Pharmacopœia, or the V or 1870 revision but neither provided much help. Both revisions prescribed a percentage content of certain alkaloids for opium and some cinchona barks, but no methods for estimating the same. In the case of opium, the morphine was to be determined by an "Official Process," but no "Official Process" was given. Scammony was required to contain 75 per cent of ether soluble material. A minimum and a maximum percentage of alcohol was prescribed for both brandy and whisky.

The British Pharmacopœia of 1864, which became an authority under the 1868 English Food and Drug Law, embodied material advancements in tests and methods of analysis. It required, for example, a percentage content of certain alkaloids for opium and some cinchona barks, with methods of analysis. Similar improvements did not come in the United States Pharmacopœia until the VI or 1880 revision, which revision was recognized as a standard by a number of the State Food and Drug Laws, enacted during the eighteen eighties. Subsequent revisions of this work embodied similar advancements of their time.

The tariff laws and the revenue derived therefrom, it is often alleged, played a more important role than did pure and safe medicines for the sick. It must, however, be conceded by all that in spite of the many handicaps encountered in the enforcement of the law of 1848 that material improvements in imported drugs resulted in the course of years.

EXTENSION OF POWERS OF THE SECRETARY OF AGRICULTURE TO IMPORTATIONS

In 1890 Congress passed a reprisal law (13) covering the importation of adulterated and otherwise, of foods and drugs. Its enforcement was vested in the Secretary of Agriculture, but required a proclamation by the President, to make it effective. The drug portion was apparently never called into action.

The appropriation act for the Department of Agriculture, March 1, 1899, authorized the Secretary to refuse entry of adulterated foods and drugs, which from an examination he had reason to believe would be dangerous to the people of the United States. Subsequent appropriation acts for the Department, contained the same authorization. In time a limited fund was made available for the work but it was restricted to foods. The above gives some idea as to the environment in which the Drug Laboratory found it necessary to adjust its early activities. More will be pointed out later. The above restriction to use the available money for port work for foods were first, because of the limited funds available, second, because drugs were already being inspected under the 1848 drug import law and third neither Dr. Wiley nor I wished to disturb the work of our friends engaged therein. But we kept more or less in touch with it. Indeed Dr. Rusby and I discussed the problems from time to time as we met. In time sufficient money was made available to establish branch laboratories at several ports by the Bureau of Chemistry. Their work was still restricted to foods, but the Drug Laboratory was permitted to have the force collect samples of drugs for examination. The records show that during the fiscal year ending June 30, 1904, a number of drug samples were taken and examined. This line of work was, however, largely crowded out, because of a study of the purity of chemicals, some of which were imported, testing out methods of analysis, the examination of proprietary medicines for Congress in connection with the proposed Food and Drug Legislation and nostrums for the Post Office Department, in the matter of the fradulent use of the mails.

In addition to the limited funds there was a decided shortage of available qualified workers, under the restrictions imposed by the Civil Service Commission. I am a staunch advocate of the principles of Civil Service, but freedom of action is often hindered, probably to the good in the long run. When we wanted to add to our force the Commission prescribed a four year University training or its equivalent which practically barred most of the College of Pharmacy graduates and others at the time, most of whom had much less training for a degree. The Commission was inflexible in this matter. Let me cite just two cases—Drs. H. H. Rusby and Charles H. LaWall. After the employment of Dr. Rusby it was found necessary to cover him into the Classified Service. After outlining the work and the salary to be paid, the Commission ruled that such a position called for a Ph.D. degree or its equivalent. It developed that Dr. Rusby had had only a short two-year course that led to a medical degree. We were at first afraid that the Commission would not pass him, the outstanding man in his field. His outstanding practical experience and contributions just got him a passing mark.

Dr. LaWall was an eminent analyst, an outstanding teacher, thoroughly versed in every branch of the drug trade, but had only a short two-year course in a College of Pharmacy. The Commission prescribed a four year University degree, or its equivalent, in his case. His outstanding practical work and contributions, as in the case of Dr. Rusby, just gave him a passing mark. Others with a longer academic training, but far less qualified, received higher grades and therefore were accorded preference to positions. This did not make for a good feeling.

Under these circumstances I urged longer courses and higher training in our Colleges of Pharmacy. I know that this advice was gratuitous and was not kindly received in some quarters, but I had experienced the mental sufferings of some of my friends and hoped to help obviate like grief to future graduates.

SERVICES OF THE BEST QUALIFIED AND AVAILABLE MEN SOUGHT AND SECURED

It was the aim of Dr. Wiley and myself to secure the services of the best qualified and available men and I believe that we were largely successful. For the port of New York City and other ports along the Atlantic Coast, we secured the services of Drs. Rusby, L. D. Havenhill and Charles H. LaWall; for New Orleans and other Gulf ports, Dr. Edsel A. Ruddiman; for Seattle, Dr. Charles W. Johnson; for San Francisco, Dr. Albert Schneider and for some of the inland ports, Professor J. O. Schlotterbeck.

The New York port was by all odds the most important, Drs. Rusby and Havenhill were put in direct charge of the drug work. Dr. Havenhill entered the service June 5, 1907, and Dr. Rusby, July 13, 1907. The former on a full time basis and the latter on a per diem basis. If the decisions of our port workers were contested, the appeals came to Washington. Certain attorneys, at the beginning, protested many of them, not so much as to their findings but on the principle that we were going beyond the law, which some at the beginning felt would not be enforced. Some of the importers contended that they could bring in anything they desired, by marking the goods as to their true character which was largely correct, but they further maintained that after the goods are once entered, they could do anything with them they desired. We held that defective drugs, though properly marked at the port of entry, could only be used in the manufacture of other preparations, by carrying along the importation markings, except when the preparation is standardized, or used for the manufacture of an active ingredient, say quinine, or the deficiency is otherwise adjusted. We even carried our position into court and were there sustained. It is true that if

an importation crossed the state lines properly marked we were helpless as to what was done within the states. That problem rested with the states.

BEGINNING OF WORK ON DRUG IMPORTS AND SOME FINDINGS

Before we were even organized for the drug work, the San Francisco force came across an importation of smoking opium, detained the shipment and sent several cans to Washington for a decision. I studied the case at considerable length. It looked like a borderline case. There was an import duty of $6.00 a pound, which raised the question as to whether the Treasury Officials might not object to its exclusion. I decided on a course of action. In time I took the case to Dr. Wiley for his decision, related the whole story as I saw it and stated my opinion and the reasons therefor, that it was a close case, but thought that under the import section of the law we could keep it out. He lifted his eyebrows, asked a few questions, then said: "I agree with you, it is a dangerous product and it is our duty to keep it out if we can—prepare instructions to that effect." Our decision set things in motion. Attorneys representing the importer, the shipper, a banker and an insurance company protested vigorously but the Treasury Officials sided with us. We stood firm. All finally agreed to abide by our decision without carrying the case either to the Secretary or the President, as was often done, during the early days. This settled the question of importing smoking opium. We believed the decision was right and in the interest of humanity. Other importations were offered, but the above set a precedent which made future action on like shipments easy. We were told that our action simply meant smuggling the stuff, which of course was not our problem.

Dr. L. D. Havenhill spent a short time in Washington to get a line on the work before going to New York, but Dr. Rusby being more or less conversant with the work at the New York port began his work directly at the port. After a little more than a month and a half given to the work, Dr. Rusby seemed to be rather pleased with the good quality of the drugs imported (14) at the time. This of course was gratifying to him because of his previous experience and the fact that the appraiser had told him that he would find things bad, but his jubilation did not last many months. It happened to be one of those anomalous affairs. At the end of fourteen months of inspection work he had a different story to tell (15).

As a prelude let me say that the "Committee on Drug Adulterations," of this ASSOCIATION, in its 1906 report (16) alleged that "adulteration as such, with intent, is quite rare." Some members did not agree with the claim and Dr. Rusby's findings at the port, at the end of fourteen months showed quite the contrary. Let me just note a few of the intentional adulterations he found and his remarks thereon. If a consignment was rejected at the New York port some importer, would brazenly threaten to reship it to a port where "we know we can get it in" and some actually did it, for a time. We were of course at the outset unable to cover all of the ports of entry as thoroughly as the City of New York. Indeed some of the adulterated or low grade drugs were imported in bond and sent to some of the inland ports like Chicago, Minneapolis, Detroit and St. Louis. Here again we were unable early to provide trained workers, but we caught up with them in time.

A consignment of five tons of powdered olive pits made its appearance. The importer alleged that it was to be used "as a filler for chicken food." We were suspicious, but could not refuse them entry. In time an examination was made of some of the powdered goods of the importer and not unexpectedly found olive pits in five samples of his powdered drugs. A large dealer on being informed that his ground belladonna root contained 50 per cent of ground olive pits, protested loudly but later found the report to be correct and learned that his miller was systematically indulging in such practices. Kamala, powdered gentian and ipecac were found mixed with goodly amounts of ground olive pits. Turmeric was adulterated with wheat starch.

In another instance an importer strenuously objected to the rejection of "belladonna root," that was all poke root and that it did not contain any belladonna root at all. Dandelion root contained 48 per cent of ash, including small stones sifted to about the size of the fragments of the root. Saffron adulterated with calendula, artificially colored and weighted. Cut dandelion root entirely substituted with chicory trimmings. Artificial benzoin. Digitalis leaves mixed with stramonium leaves. Maracaibo bark substituted for cinchona bark. Senna leaves broken, 30 per cent of ash. Belladonna leaves containing from 50 to 80 per cent of stems and fruit. Not much adulteration here that is not intentional. Relative to the results in this report Dr. Rusby says: "The importance of this demonstration can hardly be over-estimated. The plea of non-intent has been in the past the strongest defense offered. It should not have been regarded as a good one, even if justified, for responsibility is fixed, with or without good intent. The evidence this day presented, of a strictly legal character, is conclusive that there is a large amount of adulteration, pursued on a systematic and scientific basis, and with the employment of expert assistance. It is a very easy, safe and agreeable thing for men to publicly claim non-intent for each other, but a very disagreeable and more or less dangerous for one to publicly charge evil intent. Pharmaceutical education has been just as guilty of false pretense and fraudulent output as has drug purveying, and the present situation is going to show the necessity of the states' making suitable provision for the thorough education of inspectors and assayers, from their preliminary education up to their technical training."

These are very positive, far reaching charges;

ones that the Chief of the Drug Laboratory would hardly venture to make, even though they were all within his knowledge and experience. He would have lain himself open to violent attacks from many quarters but he knew that they were all true and the half had not been told.

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(13) U. S. Stat. at L., 26 (1890), 414.
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(15) *Ibid.*, 56 (1908), 783.
(16) *Ibid.*, 54 (1906), 328.

Book Reviews

The Nature of the Chemical Bond and the Structure of Molecules and Crystals, by LINUS PAULING. 2nd Edition. xvi + 450 pages. Cornell University Press, Ithaca, N. Y., 1940. Price, $4.50.

This book is a clear explanation of the different ways in which atoms are held together to form molecules. It takes up the structures of many inorganic and organic compounds in detail and, in so doing, it gives the results of electron diffraction measurements and descriptions of a considerable number of x-ray analyses of crystals. This, the second edition, contains 21 pages more than the first largely because of the addition of two sections and the discussion of some newly determined structures. The book is of particular interest because, although it outlines the ideas involved in the quantum mechanical treatment of valence and structural chemistry, it describes the new developments in a thorough and satisfactory manner without resorting to the use of higher mathematics.—A. G. D.

Annual Review of Biochemistry. Volume IX. JAMES MURRAY LUCK and JAMES H. C. SMITH, Editors. 744 pages. Annual Reviews, Inc., Stanford University P. O., Calif., 1940. Price, $5.00.

This, the ninth volume of the series, covers the scientific contributions made during 1939. In addition, reviews on the following topics have been included: plant pigments, biochemistry of the lower fungi, biochemistry of malignant tissue, organic acids of plants, clinical application of biochemistry, soil microbiology and the application of microchemistry to biochemical analysis. Three new topics are presented—the biochemistry of the viruses, application of radioactive indicators in biology and insect biochemistry. The volume contains both author and subject indexes.—A. G. D.

Kingzett's Chemical Encyclopædia. Revised and Edited by RALPH K. STRONG, Ph.D. D. Van Nostrand Company. 6th Edition. 1940. 1088 pages. $14.00.

This, the sixth edition of Kingzett's Chemical Encyclopædia, presents a book considerably enlarged in size and contents over previous editions. Reference is made to many new industrial solvents and the section on chemical engineering has been expanded to include a consideration of materials, energy, apparatus and economics. The section on bacteria has been brought up to date, the section on coal carbonization has been completely rewritten and the sections on proteins and vitamins have been enlarged. A new feature of the book is its tabulation of production and imports by countries of widely used chemical commodities. One of the most valuable features of the book is believed to be its inclusion of many commercial names of chemicals, thus making it a commercial index to chemicals as well as a dictionary. The volume should prove to be useful as a reference work for every day use by pharmaceutical chemists and pharmacists.—A. G. D.

The AMERICAN PHARMACEUTICAL ASSOCIATION has received a copy of the annual report for the year 1939 of the Egyptian Government Central Narcotics Intellegence Bureau for the Alexandria, Port Said, Suez, Cairo and Provincial Branches. The report covers activities with respect to seizures on land and sea in the territory within the above jurisdiction. The narcotics named are Indian hashish, heroin, opium, etc. The records of the offenders are searched for prior offences and these are made of record. Information is also sought as to the history of the individuals—their trade or profession, habits, frequency of imprisonment, dosage amounts of the narcotics consumed—the dosage, if chemists are required to discover the addiction, determining the legal phase. Interesting illegalities of cases enter into the reports. The illegal drugs are hidden in various ways—in the stomachs of camels—illustrations show tins containing opium, hashish, etc.—E. G. E.

Reference Book of Inorganic Chemistry, by LATIMER and HILDEBRAND. The Macmillan Company. 4th Edition. $4.00.

The fourth edition of this reference book follows the third by only two years, which is indicative of

its popularity. The new edition contains 120 pages more than the preceding one, but this is not due so much to an increase in the contents as to the use of larger and more legible type. Revisions have been made in the values of many physical constants, the chapter on atomic nucleus has been completely rewritten and other minor changes have been made. In the appendix, two little used tables have been dropped and replaced by tables on the structure of molecules and ions and on the co-valent bond energies and atomic radii. The book is recommended as being useful as a handy reference on inorganic chemistry.—A. G. D.

Qualitative Analysis and Chemical Equilibrium, by T. R. HOGNESS and WARREN C. JOHNSON. xii + 417 pages. 5½ x 8⅛. 1937. New York: Henry Holt & Co. $2.75.

This book, intended as a textbook of qualitative analysis, aims to teach the underlying reasons of the reactions involved as well as methods of qualitative testing. Part I of the books deals with the general properties of solids, liquids and solutions, velocity of reaction and equilibrium, solubility product, colloids, ionization of water, hydrolysis, amphoteric substances, oxidation and reduction and sulfide precipitations. Part II deals with group precipitations in the usual order, except that the alkali metals are studied first, then the silver group, etc.—A. G. D.

The Chemistry and Toxicology of Insecticides, by HAROLD H. SHEPARD. iii + 383 pages. 8⅛ x 10¾. 1940. Minneapolis: Burgess Pub. Co. $4.00.

This volume is intended as a textbook for students of economic entomology, but in reality, it is a compilation of a great deal of practical information on insecticides and should, therefore, become of increasing value to pharmacists whose business in this field is increasing. The contents are divided into chapters dealing with arsenical stomach poisons and non-arsenical stomach poisons such as fluorides, thallium, lead, mercury, copper and zinc salts, tartar emetic and borax. Contact poisons are also dealt with. Among these are sulfur, selenium and copper compounds, and substances used to increase their effectiveness, plant derivatives, insect fumigants, attractants and repellants and methods of testing fly sprays.—A. G. D.

The Badianus Manuscript (Codex Barberini, Latin 241—Vatican Library—An Aztec Herbal of 1552). Translated and annotated by EMILY WALCOTT EMMART. xxiv + 341 pages. 9½ x 12¼ in. 1940. Baltimore: The Johns Hopkins Press. $7.50.

The Badianus Manuscript is a work on the medical lore of the Central American Indians. It was originally inscribed by an Indian physician in Aztec and translated by another Indian into Latin. The Aztec portion from which the present translation was made was written by Martinus de la Cruz, a native medical teacher, and the botanical portion was translated by Joannes Badianus. The present volume is an English translation of the latter with annotations by the author. It gives a good picture of certain aspects of Aztec medicine practiced in the days of the Conquistadores. The book contains 117 color plates which reproduce in full size the illustrations in the original book. This is followed by a translation of the Latin chapter and descriptions of drugs and plants with accompanying translations. Explanatory comments by the author add to the value of the work. There is a comprehensive Aztec index followed by interesting botanical, materia medica and disease indexes.—A. G. D.

Chemistry and Medicine. Papers Presented at the Fiftieth Anniversary of the Founding of the Medical School of the University of Minnesota. Edited by MAURICE B. VISSCHER. 296 pages. The University of Minnesota Press, Minneapolis, Minn., 1940. Price, $4.50.

This volume is a compilation of papers presented at the fiftieth anniversary of the founding of the Medical School of the University of Minnesota. The papers are arranged in four groups and deal with the progress in the application of physical chemistry to medicine, recent investigations in metabolism, some aspects of immunity in chemotherapy and some approaches to the nervous control of the organism. In the first section, there are papers dealing with the colloid structure of membranes, osmotic work in living systems and the function of the kidney in concentrated solutes. The second part deals with the more important subjects of vitamin research, the importance of fats in the diet and heparin. Papers on immunity and chemotherapy constitute the third part and the last part of the book presents the essential facts relating to nervous control in animals and the methods by which nervous action may be analyzed. All of the papers are supplemented by extensive bibliographies.—A. G. D.

Mineral Metabolism, by ALFRED T. SHOHL, M.D., Research Associate in Pediatrics, Harvard University. Published by Reinhold Publishing Corporation, New York. Price $5.00. American Chemical Society series of Scientific and Technologic Monographs.

It is stated that the main purpose of the book is to describe the role of the minerals in the structure and function of the human body. The author has given much study to the subjects which is shown by the extensive bibliography appended to the discussions. Chapter I deals with the scope of mineral metabolism; the elements which occur in biological material are classed in five groups—Definition of Mineral Metabolism, Relations of Minerals to Water; Relation to Various Fields of Biology, to Animal Nutrition, etc. References under the

Bibliography end the Chapters. Chapter II is devoted to the mineral composition of the body; the first "Body Water," its distribution, blood, cerebrospinal fluid and individual minerals. Secretions and excretions are discussed in Chapter III. Tables and figures add value to this division. Internal Secretions, glands, are considered in Chapter IV. Total base, chloride, ammonium, bicarbonate, sodium and potassium are the subjects of Chapter V. Chapter VI lists calcium and magnesium. Chapter VII, on phosporus, ends with a list of 55 references; Chapter VIII is given to sulfur. Critical reviews of sulfur metabolism have been made by Lewis and by Vigneaud and Dyer. Chapter IX treats of iron in the body and in various forms. The divisions are given to functions, iron compounds in the body, differentiation of organic and inorganic compounds, quantitative distribution, metabolism, hemaglobin, balance studies and requirements and sources of iron in food. Iodine is treated in Chapter X, iodine compounds in the body, metabolism, disorders of the thyroid gland, sources of iodine and supplements in food. Chapter XI considers a number of elements which are present in small quantities, among them aluminum, arsenic, bromine, cobalt, nickel, copper, fluorine, manganese, selenium, silicon, zinc; all of them are listed in the bibliography of 201 references. Chapter XII is headed "Water Metabolism" and the subject is considered under 89 titles and various phases—functions, excretion, requirement and control. Chapter XIII treats of Anion—Cation Relationships and Chapter XIV of mineral intakes, balances and requirements. Indexes conclude the volume of nearly 400 pages.—E. G. E.

Handbook of Mathematical Tables and Formulas, by RICHARD STEVENS BURINGTON, Ph.D., Associate Professor of Mathematics at Case School of Applied Science, Cleveland. 275 pages, index included. Published by Handbook Publishers, Inc., Sandusky, Ohio. Second Edition, 1940.

This book has been compiled for the needs of students and workers in the field where mathematical computations are required. The first part of the book has a summarization of fundamental formulas and theorems of algebra, trigonometry, analytical geometry, calculus and vector analysis along with a comprehensive table of derivatives and integrals of calculus. A total of four hundred and thirty-four integral equations are given. In the second part of the book is given the usual logarithms and trigonometric tables to fourth and fifth places, with explanation as to their use; tables of natural logarithms of exponential and hyperbolic functions, reciprocals, logarithms of Gamma functions and factorals. An improved table of squares, cubes, square roots and cube roots has been incorporated in this edition. This Handbook forms the appendix of Lange's "Handbook of Chemistry" especially prepared for chemist and physicist. The usefulness of Burington's Handbook is highly regarded as an essential to obtain speed and accuracy in mathematical computations.—E. C. BEELER.

Modern Cosmeticology—The Principles and Practice of Modern Cosmetics, by RALPH G. HARRY, F.I.C., M.R.I.P.H., F.R.M.S., A.R.P.S., 1940, Publishers, Chemical Publishing Co. Inc., 148 Lafayette St., New York City. Price, $5.00.

The author has endeavored to deal with the subject by making use of research which has required the application of scientific thought. He has given attention to questions which may arise by the addition of ingredients that are added for emulsification or permanency and also the histology of the skin and hair that may be injuriously affected or effected. The publication contains an important collection of photomicrographs showing the structure of hair, skin, nails; studies underlying the principles of the uses are presented. The author states that eye lotions are seldom fully discussed in books and periodicals. While this statement is no doubt true, there are very good reasons for being exceedingly careful of the limitations in their inclusion and substantial reasons for greater caution relative to the dangers of self-medication. It is well enough to improve the formulas and limit them, but it is equally desirable to discourage self-medication and treatment without consulting the physician. In many instances a warning from the pharmacist based on his experience is a safeguard. Evidently the author has recognized the dangers and applied his deductions for safety. It is a fact that many of the users are guided only by appearance at the time of application, but the pharmacist's responsibility does not always end there. The book serves a useful purpose as a guide to the manufacture and use of cosmetics. Possibly more should have been said about too frequent applications which may interfere with the natural processes.—E. G. E.

Specific and Special Reactions for Use in Qualitative Analysis, with Particular Reference to Spot Test Analysis, by F. FEIGL. Translated by Ralph E. Oesper. Third German edition revised. ix + 192 pages, 15.5 x 23 cm. Elsevier Publishing Co. and Nordeman Publishing Co., New York, N. Y., 1940. Price, $3.50.

This volume is an English translation of the third German edition of "Qualitative Analyse Mit Hilfe von Tüpfelreaktionen." It is stated in the preface that the translator has taken the opportunity of including some material for the fourth German edition which it is expected will be published shortly. The new material consists primarily of additional references to the literature to bring the work up to date—48 new references are given. The book presents a new approach to the subject of qualitative analysis and will no doubt be welcomed by workers in this field.—A. G. D.